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FIVE TYPES OF SO-CALLED BRONCHIAL ADENOMA

A Histopathologic Study

CAPTAIN MACHTELD E. SANO

AND

LIEUTENANT COLONEL RICHARD MEADE Jr.

MEDICAL CORPS, ARMY OF THE UNITED STATES

FIVE CASES have been chosen to illustrate the variation of the histopathologic structure and of the cellular characteristics of the so-called bronchial adenoma. Much has been written in recent years of the cellular origin of bronchial adenoma, of its relationship to embryologic rests [1] and of its behavior.[2] Even today the controversy as to its potential malignancy is still a point of debate.[3] A brief review of the anatomy of the region in which the tumor occurs, i. e., the larger bronchi, will clarify the discussion.

The larger bronchi are lined by several rows of cells: basal cells, intermediate cells, cuboidal cells and ciliated cells. Underlying these layers is a musculoelastic stroma. The crescent cartilages of the bronchi differ in size and shape. They have been described in detail by Miller.[4] In the bronchi the membranous interval separating the cartilages from each other is mesial. Glandular structures can be found in these intervals. These glands form part of a cluster lying either behind or in front of the bronchial cartilages. Examination of many normal bronchi, as well as of bronchi in cases of diseases of the lungs, shows a similar distribution. The cells lining the mucous glands are relatively tall and triangular, with the rounded tip of the triangle directed toward the lumen. The cytoplasm of these cells is either finely granular or vacuolated and takes a pale basophilic stain. The serous cells have a darker, sometimes finely granular cytoplasm. The component cells of these glands are either mucoid or serous, with the mucoid variety usually predominating

From the Chest Service and the Department of Pathology, Kennedy General Hospital, Memphis, Tenn.

1. Graham, E. A., and Womach, N. A.: J. Thoracic Surg. **14**:106, 1945.
2. Clerf, L. H., and Bucher, J. C.: Ann. Otol., Rhin. & Laryng. **51**:836, 1942.
3. Jackson, C. L.; Konzelmann, F. W., and Norris, C. M.: J. Thoracic Surg. **14**:98, 1945.
4. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1943.

by far. However, occasionally the cell types are more evenly distributed and one may see dark-staining crescents at the terminal portion of the gland. These are sometimes referred to as the demilunes of Giannuzzi.[5] These serous crescents may not be present in the glands of some lungs. The nuclei of these cells are centrally placed, while in the mucous cell the nucleus is at the base. In the isthmus of the gland the basal cell layer can be clearly visualized. These basal cells have small round nuclei rather than the oval type which one usually finds in this type of gland in other parts of the body. The interstitial stroma is delicate, sometimes hard to identify. In the distal portions of the larger bronchi the glandular structures become progressively smaller and less numerous until in the terminal portions and in the bronchioles they are entirely absent.

The cells which one sees in the bronchial adenoma resemble the different component cells of the bronchial glands and are found in the same anatomic locations. The predominating tumor cell type may vary, however, and this is true in each of the cases to be reported. While in certain parts of the tumor a tendency toward acinous arrangement and the cell relationship may be preserved, in other parts this may be completely lost and the tumor be identical with an undifferentiated cell carcinoma. A detailed description of the cellular structure of the tumor is made in the report of each case. In the normal bronchial glands the mucicarmine stain was not always typical. It was negative in all the tumor sections, so that no differentiation into cylindromas was made.[6]

Local removal of bronchial adenoma is not advocated (Graham and Womach[7]), though a few are still in favor of this mode of treatment (Jackson and co-workers[8]). As the first case and figure 1 illustrate, the tumor, pathologically speaking, does not recur; it simply continues to grow. Only the clinical symptoms recur.

REPORT OF CASES

CASE 1.—A man aged 24 was admitted to the Kennedy General Hospital in May 1944 for study. In October 1943 he had atypical pneumonia, which involved first the middle and then the upper and lower lobes of the right lung. The sputum contained pneumococci. A similar attack occurred in January 1944. In June 1944 cough and fever developed, and roentgen examination showed atelectasis of the lower lobe of the involved lung. On bronchoscopy an adenoma of the left

5. Maximow, A. A., and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1937, p. 35.

6. (*a*) Adams, W. E.; Steiner, P. E., and Block, R. G.: Surgery **11**:503, 1942. (*b*) McDonald, J. R.; Moersch, H. J., and Tinney, W. S.: J. Thoracic Surg. **14**:445, 1945.

7. (*a*) Womach, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938; (*b*) Graham and Womach.[1]

primary bronchus was seen and the diagnosis was confirmed by biopsy of the tumor tissue. A left pneumonectomy was performed in September 1944. The patient's recovery was uneventful, and he was discharged from the hospital in December 1944.

Pathologic Examination.—In the left lung was a pedunculated tumor, which arose from the spur at the bifurcation of the left main bronchus. The tumor completely obliterated the upper bronchus. Mechanically it could at times just as well have obliterated the lower branch. The mucosa proximal and adjacent to the pedicle was slightly roughened and firm. Cross section of the tumor and part of the bronchus (fig. 1) showed the pedunculated tumor as it arose from the bronchus. It could have been removed through the bronchoscope, with relief of symptoms. Microscopic examination of the pedicle would not have revealed

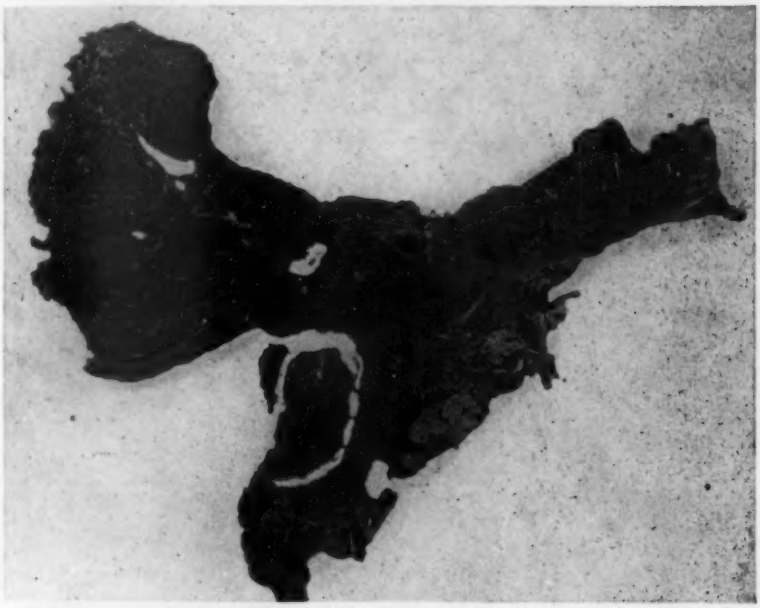

Fig. 1.—Pedunculated tumor in case 1. The glands distal and proximal to the base of the peduncle contain tumor cells. × 4.5.

the presence of tumor. Actually the tumor would have recurred. As may be seen in figure 1, the glandular structures both proximal and distal to the pedunculated tumor contained many tumors cells. Figure 2 *A*, a higher magnification of this area, illustrates the glandular structures and the tumor. It is true that the mucosa proximal to the pedicle was slightly roughened, but the mucosa distal appeared intact. This distal area of the tumor could not have been seen even on closer scrutiny after the bronchus had been opened. In the same way a cluster of glands behind the cartilage contained the tumor cells. Local removal of the tumor present, as well as of the tumors which would have arisen on recurrence of growth, could only have alleviated the condition, while during this period of temporization the tumor behind the bronchus would have invaded the surrounding tissue. Pneumonectomy would have become a necessity but under much less favorable conditions. Whether these different sites of tumor in these glandular

structures developed independently would be difficult to determine. Suffice it to say that a pedicle-free tumor does not preclude absence of tumor in the remaining portion of the bronchus. Small islands of cartilage and bone were seen in this tumor. Cases 1 and 2 (fig. 2 *A* and *B*) illustrate, perhaps better than any of the

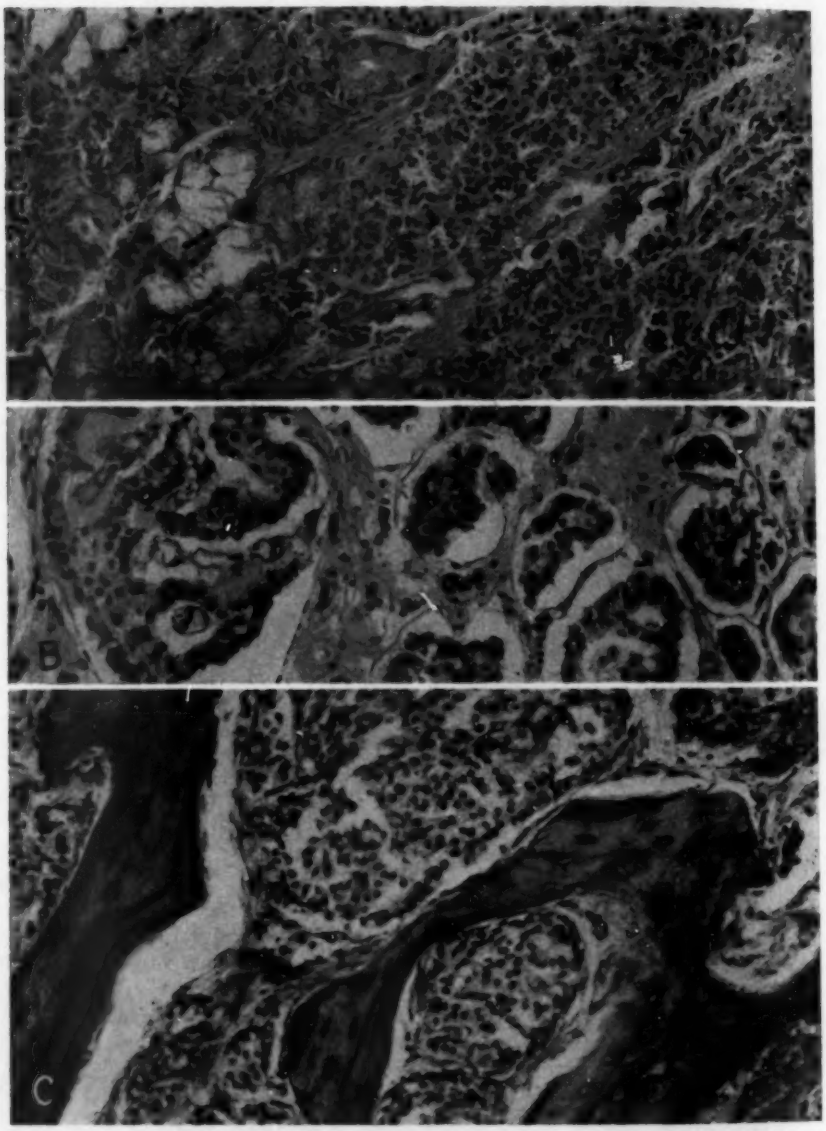

Fig. 2.—*A*, tumor cells distal to the pedunculated tumor shown in figure 1. × 200. *B*, acinous arrangement of the tumor cells in case 2. *C*, bone occurring in bronchial adenoma in case 2. Bone is frequently found in specimens of bronchial adenoma. Abnormal bone formation in the lung is not unusual. × 200.

others, the intimate relationship which exists between the tumor and the glandular structures of the bronchi without actual neoplastic invasion of the intervening tissue.

CASE 2.—A 22 year old man was admitted because of bronchial obstruction. An obstructing tumor was seen on bronchoscopy, but biopsy failed to reveal definite evidence of tumor. Excision of the lower lobe of the right lung was performed successfully, and the patient had an uneventful recovery.

Pathologic Examination.—A tumor measuring 3 by 2.5 cm. was found arising from the wall of the main lower branch bronchus. It compressed the bronchus, causing almost complete obliteration of the lumen. The mucosa of the bronchus was intact and grossly not invaded by tumor, although it was edematous and congested. On section the tumor was found to be formed by soft tissue and bone. In figure 2 *B* the acinous arrangement of the cells of this tumor is evident. The cells with the dark-staining nuclei are identical with those seen in figure 2 *A* of case 1. The pale cells are like the cells of the mucous glands and those seen in the isthmus of the glands. Note that when both types of cells are found together, the paler cells are always central and the small round cells peripheral. The nucleus of the pale type, has delicate chromatin markings and a small pale nucleolus. The cytoplasm is pale, taking neither the basophilic nor the acid stain too well. Mucicarmine stain is not more revealing. As can be clearly seen in figure 2 *B*, the acini sometimes contain ovoid bodies with deep-staining nuclei and a delicate but well outlined vacuoloid structure. These bodies do not resemble oncocytes.

There are, therefore, two types of epithelial cells in bronchial adenoma: the clear tumor cells which have a close resemblance to the secretory cells of the bronchial glands, and the deep-staining round cells almost identical with the basal cell layer. In different tumors and frequently in different areas of the same tumor, as in case 2 (fig. 2 *B* and *C*), one or the other type of cell predominates. The stroma is prominent and evenly distributed in figure 2 *B* (case 2) and scant in figure 2 *C* (same case).

Whenever there is predominance of the small round cells, there is lack of the tendency to acinous arrangement. This feature is clearly seen in figure 3 *A* of case 3.

CASE 3.—A 29 year old man had presented symptoms since August 1943. On the roentgenogram there was an infiltrative process, which cleared after penicillin therapy was begun, but there remained thin-walled cysts. Biopsy material removed elsewhere was diagnosed as infiltrative carcinoma. Tissue removed on bronchoscopy in this hospital was diagnosed here as bronchial adenoma. Pneumonectomy on the left side was performed, and the patient had an uneventful recovery.

Pathologic Examination.—The bronchus leading into the upper lateral portion of the lower lobe of the left lung was obliterated by a soft, pink tumor measuring 1.2 by 1 cm. Its base measured 0.9 cm. The tumor had invaded the anterolateral side of the bronchus, forming on the other, outer wall of the bronchus a small nodule, which measured 0.2 by 0.3 cm.

Microscopic Examination.—This tumor (fig. 3 *A*) had much less tendency toward acinous arrangement than the previous ones. The small round cells predominated. There was little stroma, and even more rare were pale cells such as those seen in case 2.

Though in general structure all these tumors were similar, and the same types of cells could be found in each one of them, it is evident that the number of cells of each type present varied considerably, and on biopsy they might even be confused, as in case 3, with undifferentiated cell carcinoma. (This tumor in a small fragment of tissue was diagnosed

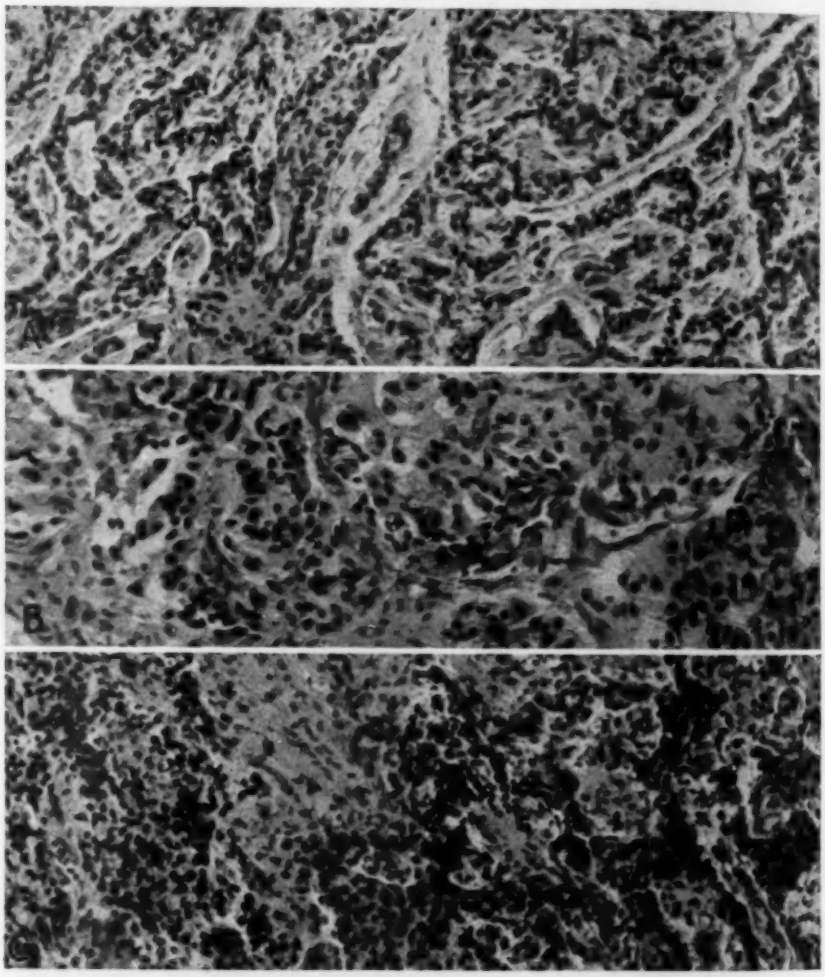

Fig. 3.—*A*, predominance of small round cells (case 3). × 200. *B*, tendency toward acinous arrangement in an area where clear cells are found (case 4). *C*, predominance of small round cells (case 4). This predominance was noted throughout the tumor. There had been widespread invasion of the entire lobe.

by some of the most able pathologists as undifferentiated cell carcinoma and later, when the whole tumor was examined, as bronchial adenoma.)

Case 4.—A man aged 51, who had no thoracic symptoms, was examined for an inguinal hernia, arthritis and chronic nephritis. During this work-up, a roent-

genogram of the chest was taken, which revealed atelectasis of the upper lobe of the right lung. Bronchoscopy revealed an obstruction of the bronchus of the right upper lobe. Biopsy material disclosed no evidence of tumor. In view of the obstruction and the patient's age it was considered that tumor was probably present in the bronchus of the upper lobe. During operation the patient ceased to breathe and could not be resuscitated. Postmortem examination showed that he had died of asphyxia secondary to an unusual amount of fairly adherent mucoid material in the bronchi and bronchioles of both lungs.

Pathologic Examination.—Almost the entire upper portion of the upper lobe of the right lung was found to be atelectatic and firm. When opened, the bronchi of this lobe showed evidence of a chronic inflammatory process. There were both longitudinal and cross striations in the mucosa, and the walls of the bronchi were thickened. One of the smaller bronchi was dilated and the mucosa destroyed, being replaced by a mass of dark mucoid material. In the immediate vicinity of this region the mucosa showed some papillary hyperplasia. The tissue surrounding this area was hard and infiltrated by tumor. The bronchi of the lower lobe were filled with a bloody mucoid exudate.

Microscopic Examination.—This tumor, as illustrated in figure 3*B* and *C*, contained both types of cells, but the small round cells predominated throughout. The tendency toward acinous arrangement had been almost completely lost except where the clear cells were found, as in figure 3*B*. Figure 3*C* illustrates the histopathologic character of the greater part of the tumor. In our opinion this is a so-called bronchial adenoma which has undergone malignant alteration, widely invading the upper lobe without, however, having produced metastases in the lymph nodes or other organs.

The last case is presented because it forms a composite picture of the 4 cases described in the preceding pages.

CASE 5.—A 35 year old woman had cough since the summer of 1944. A roentgenogram showed evidence of disease in the lower lobe of the right lung. On bronchoscopy a tumor was seen obstructing the bronchus of this lobe. Tissue removed from this tumor was diagnosed as adenoma. Pneumonectomy was done on the right side, and the patient had an uneventful postoperative recovery.

Pathologic Examination.—While in case 2 the tumor appeared to arise in the wall of the bronchus, leaving the mucosa intact, and in case 1 it was frankly pedunculated, this tumor resembled neither. It appeared to arise in the wall, penetrating the mucosa over an area measuring 1.4 by 0.9 cm. and completely obliterating the lower branch bronchus. The tumor likewise penetrated the outer wall of the bronchus into the lung parenchyma without, however, invading it, the tumor on this side being well encapsulated. Of all the specimens of bronchial adenoma, this one was by far the hardest, and this was found to be due to bone.

Microscopic Examination.—This tumor is of great interest because in it we found the different patterns described in the other 4 cases. A large part of the tumor was identical with that in case 1 (fig. 2*B*) and that in case 3 (fig. 3*A*) and another part again with that in case 2 (fig. 2*C*) except that here and there were less pale cells and fewer bone spaces with tumor. One area when seen isolated was diagnosed as undifferentiated cell carcinoma and resembled the small cell areas seen in case 4 (fig. 3*B* and *C*). On the other hand, this tumor was well delimited and did not invade the surrounding parenchyma.

Womach and Graham [7a] promulgated the theory that tumors of the type described arise from undeveloped bronchial buds. They expressed the belief that the presence of cartilage and bone in these tumors, though not always found,[8] supports their hypothesis. On the other hand, Mallory [9] and Stout [8] have expressed the opinion that this is insufficient evidence. We tend to agree with them.

Of all the organs, the lung is the one exposed most to chronic infection with secondary inflammatory reaction. Also it is not unusual to find bone [10] arising at the extreme end of the cartilage crescents, especially if the inflammatory process has invaded the intercartilaginous spaces. Study of multiple sections of the upper ends of the larger bronchi in diseased lungs confirms this, and it is not unusual to find early calcific infiltration, osteoid tissue or bone. This is especially true when there is a long history of chronic infection.

Oncocytes, small round cells described by Hamperl,[11] unquestionably must be considered as a possible origin of these tumors. In case 1, as well as in case 2, several small cells with acidophilic cytoplasm were found, but they were not characteristic of the oncocytes, nor did they resemble the tumor cells. A study of the bronchial glands and the mucous glands in 50 cases of pulmonary diseases necessitating lobectomy showed that in about one fifth oncocytes could be found. In 1 case of cylindric bronchiectasis the mucous and the serous glands showed unusual secretory activity, and the mucoid material was packed with oncocytes, yet no bronchial adenoma could be demonstrated in this lobe. Several months later a specimen was sent to the laboratory which had been collected in the routine postoperative check-up of cases. Examination of the slide showed such a striking picture as to remind us of the previous case. When the slides of the previous case were reviewed, it was found that we were dealing with one and the same patient. These oncocytes do not have any resemblance to any of the cells found in any of our 5 cases of bronchial adenoma. When present in large number as in case 4, the cells were also found in the mucoid glands, yet these cells could not be confused with the tumor cells.

SUMMARY

Case 1 illustrates the inadequacy of local removal of pedunculated bronchial adenoma. This case also shows the intimate relationship of the bronchial adenoma to the mucous and the serous glands (fig. 2*A*).

8. Stout, A. P.: Arch. Path. **35**:803, 1943.
9. Mallory, P., cited by Stout.[8]
10. Wells, H. G., and Dunlap, C. E.: Arch. Path. **35**:420, 1943.
11. Hamperl, H.: Virchows Arch. f. path. Anat. **282**:724, 1931.

The presence of bone is described in 3 cases. It is considered insufficient evidence to support the hypothesis that such tumors originate from undeveloped bronchial buds.

All these tumors are considered to be "mixed", the two component cells being of epithelial origin.

The tumors described were found to have invasive tendencies and were considered as potentially malignant. In one, cancerous alteration had occurred. If only for this reason, radical surgical removal, when feasible, is advocated.

Oncocytes (Hamperl [11]) have been found in 3 of these cases, but in none were they found so profusely as in the case of bronchiectasis in which no adenoma was found. These cells do not resemble tumor cells.

PRIMARY FIBROSARCOMA OF THE HEART WITH A VERTEBRAL METASTASIS

EPHRAIM WOLL, M.D.
AND
AUSTIN L. VICKERY, M.D.
BOSTON

ALTHOUGH primary tumor of the heart is rare in the experience of any one laboratory, the cases recorded in the literature form an impressive collection. Particularly rare is primary tumor of the heart with distant metastasis. Such a case is now reported, with a brief discussion of the clinical and pathologic aspects.

REPORT OF A CASE

A Russian-born, 47 year old housewife was first seen at the Peter Bent Brigham Hospital, Boston, on Aug. 17, 1944. The complaint was a steady non-radiating pain in the right scapular area with an ache in the right arm, numbness of the right palm, and stiffness and weakness of the fingers of the right hand. These symptoms had developed progressively since six months before admission when the patient had a brief illness characterized by cough with yellowish sputum, weakness, fatigability, and pains in the knees on walking. The cough persisted but became nonproductive. A lump had been removed from the left breast in 1916. No recurrences, metastases or other complications followed.

On examination the temperature was 100.2 F. (rectal), the pulse rate 112, the respiratory rate 20, and the blood pressure 100 systolic and 60 diastolic. The patient was a well developed, moderately obese, middle-aged woman. There was definite clubbing of fingers and toes. (The patient stated that the clubbing had been present as long as she could remember.) There was no tenderness of the spinal column. No abnormal masses were felt in the thorax, the abdomen or the extremities. The heart had a thrusting apical impulse just outside the left midclavicular line with a grade 2 apical systolic murmur and an exaggerated M_2. P_2 was greater than A_2. No diastolic murmurs were heard.

The red blood cell count was 5,200,000; the hemoglobin content, 12.8 Gm. per hundred cubic centimeters; the sedimentation rate (Wintrobe), uncorrected, 50 mm. in one hour. The white blood cell count was 11,800, with 80 per cent polymorphonuclears. Repeated blood cultures yielded no growth. The electrocardiograms showed only sinus tachycardia. A roentgenogram of the chest (August 19) revealed no cardiac enlargement, and no enlargement of the left auricle was seen by fluoroscopy. The lung markings were increased. A roentgen examination of the spinal column showed "slight scoliosis of the mid-thoracic part with convexity toward the right and a localized hypertrophic

From the departments of pathology of Peter Bent Brigham Hospital and Harvard Medical School.

spurring anteriorly and laterally between the seventh and eighth thoracic vertebrae, with eburnation of the opposing margins. The intervertebral space is preserved."

The temperature ranged between 98.6 and 99.6 F. (oral); the pulse rate, between 80 and 100; the respiratory rate, between 20 and 30. On the fourteenth hospital day sulfadiazine therapy (6 Gm. a day) was begun and continued for fourteen days, without effect on the tachycardia or the fever. September 7, instillation of lipiodol, 40 per cent iodine, N. N. R., was attempted unsuccessfully. Following this, the temperature rose through the day to 103 F. and the pulse rate to 140, while the blood pressure fell to 90 systolic and 50 diastolic. That afternoon many rales were heard over both lung fields. The white cell count rose to 14,500. A roentgenogram of the chest (September 8) showed marked congestion about the hili, and a small amount of fluid at the base of the right lung. The heart was not enlarged. Over the following three days the patient improved. The rales cleared, and the temperature regained its previous level. A roentgenogram of the chest (September 18) showed a somewhat globular heart 4 per cent above average size by heart-weight ratio. There was less pulmonary congestion and no pleural fluid.

Second Admission (Nov. 6 to 22, 1944).—The backache had become more severe, keeping her awake nights, and the weakness of the right arm had increased. The numbness of the right palm now extended to include the second and fifth fingers. The nonproductive cough persisted. The patient had not apparently lost weight but appeared chronically ill. The fingers were perhaps more clubbed than before. The apical murmur was now louder.

The red blood cell count was 3,700,000, the hemoglobin content 10 Gm. per hundred cubic centimeters, the sedimentation rate 54 mm. in one hour and white blood cell count 11,000 with 85 per cent polymorphonuclears. Seven blood cultures yielded no growth. A roentgenogram of the chest (November 7) showed it to be enlarged 6 per cent above the average and globular in shape. The lungs showed coarse markings.

The pulse rate fluctuated between 80 and 100 per minute and the temperature between 98 and 100 F. (oral). The symptoms were unexplained and were poorly relieved by acetyl salicylic acid and codeine.

Third Admission (Jan. 11 to 21, 1945).—The patient was bedridden and was taking analgesics continuously for pain of the right shoulder and arm. She preferred an orthopneic position because the pain was less severe rather than because of dyspnea. She could scarcely move her right arm.

At this time her temperature was 101 F. (rectal), the pulse rate 128, the respiratory rate 26 and the blood pressure 118 systolic and 80 diastolic. The skin was pale, sweaty and cyanotic. There was marked atrophy of the interosseous and thenar muscles of the right hand. The heart was now enlarged to percussion, and both loud systolic and diastolic murmurs were heard at the apex. The liver was palpable and tender. There was moderate edema of the sacral area and of the legs. Rales were heard at the bases of the lungs. Clubbing of the fingers was now more marked on the right than on the left.

The hemoglobin was 9.6 Gm. per hundred cubic centimeters; the white blood cell count was 14,000, with 70 per cent polymorphonuclears. Repeated blood cultures yielded no growth. A roentgen examination of the spinal column showed "the transverse process of the first thoracic vertebra to be thin, hollow, as if filled with expanding tumor." The heart was enlarged 15 per cent. Pulmonary congestion was marked. A slight amount of fluid was present in both costophrenic

angles. The mediastinum appeared normal. The patient became progressively more cyanotic and dyspneic. Her temperature ranged between 98 and 102 F. and the pulse rate between 100 and 130. The sputum became bloody, and a roentgenogram of the chest (January 11) showed definite clouding of both lungs, with little aerated lung remaining. The white blood cell count rose to 41,900, with 94 per cent polymorphonuclears. Despite administration of digitalis and positive pressure oxygen, the patient died.

Necropsy (thirteen hours after death).—Permission to examine the head was not granted. Only the significant findings will be described at length. These were found in the heart and the vertebral column.

(*a*) Heart: The heart weighed 310 Gm. It was enlarged, moderately hypertrophied, and dilated, especially on the right side and in the left atrium. In the opened heart as seen from above, the lumen of the mitral valve appeared to be completely occluded by a pale yellow, firm, slightly lobulated, polypoid structure, firmly attached by a broad base to the auricular surface of the posterior leaflet and the adjacent atrial wall (fig. 1 *A*). With some difficulty, the index finger could be passed from below between the tumor and the anterior leaflet of the valve. Passage from above was possible but was considerably more difficult. The over-all dimensions of the growth were: 4 cm. wide, 2.5 cm. high and approximately 3 cm. thick at the base. The external surface was alternately smooth, firm and yellow and pale pink, granular and friable. On cross section the growth fused imperceptibly with the endocardium and formed a coarse, pale gray, homogeneous, firm mass with poorly delineated, radially arranged bands separating adjacent peripheral lobulations.

The ventricular surfaces of the leaflets were smooth and glistening. The chordae tendineae were thickened but not fused or distorted. The papillary muscles were hypertrophied. The other valves of the heart showed no significant changes except for slight thickening, reduced translucency and fenestration of the pulmonary cusps. The endocardial surface of the left atrium was strikingly thickened and corrugated. Elsewhere it was thin, light gray and translucent. The myocardium of both the right and the left ventricle was hypertrophied. There were no other areas of tumor, thrombosis or scarring. The coronary vessels were pliable and of normal caliber throughout. There were no anomalies.

Small blocks of the tumor were immediately fixed in Zenker's fluid. The heart was fixed in 10 per cent solution of formaldehyde U. S. P. in toto, and several blocks were taken after fixation. The sections were stained in eosin and methylene blue, hematoxylin and eosin, phosphotungstic acid–hematoxylin, and aniline blue. They showed relatively acellular, irregularly arranged bundles and whorls of collagenous fibers with variably sized and shaped connective tissue cells with the appropriate stains (fig. 1 *B*). Most of these cells appeared to be mature fibroblasts; others had plump spindle-shaped vesicular nuclei with abundant granular cytoplasm or delicate extracellular fibrillar projections. Still others were stellate or round with large hyperchromatic nuclei. There were also a few large tumor giant cells with amorphous dark nuclei. There was no evidence of cross striations, centrioles, intracytoplasmic fibrils or other staining qualities consistent with a tumor of muscle cell origin (Wolbach [1]). There were no myxomatous or angiomatous features. On the basis of the foregoing observations the diagnosis of fibrosarcoma was made.

1. Wolbach, S. B.: Anat. Rec. **37**:255, 1928.

The most active cell proliferation was seen at the periphery and the base of the growth. The center was composed of markedly hyalinized tissue. Invasion of the subendocardium was local and appreciable only microscopically. Most of

Fig. 1.—*A*, tumor seen from above through the opened left atrium. Note the strikingly occlusive character of the growth.

B, microscopic view of the primary tumor. Note the lobulation, the whorled character of the fascicles, the abundance of collagen and the wide variation of cell size and shape. (Eosin-methylene blue; × 143.)

the tumor was seen to grow over a much thickened but otherwise undisturbed endocardium. The external surface was covered in part by a thin layer of platelets and fibrin In a few places this covering and the subjacent part of the tumor were broken down.

(*b*) Vertebral Column: At the level of the first thoracic vertebra, directly beneath the bifurcation of the innominate artery, there was an ovoid smooth resilient mass measuring 5 cm. in length, 2.5 cm. in width and 1.5 cm. in height. The superior surface was encapsulated, smooth and free, while the base was intimately bound to the vertebral column. The tumor covered the latter and extended laterally to the right over the attachment of the second rib. On cross section the mass was seen to be a light gray homogeneous firm tissue enclosed by a fascial capsule. On pressure a small amount of dry friable material was expressed from the center. The tumor invaded and destroyed the underlying vertebral bodies and distinctly compressed the spinal nerve roots as they issued out of the spinal canal.

Blocks of the tumor were fixed and stained as described in an earlier paragraph. Microscopic examination showed it to be essentially identical with the one seen in the heart (fig. 2). The difference was in the much better preservation and more active proliferation of the cells of the former.

The seventh thoracic vertebra showed the characteristic lipping of hypertrophic arthritis. On gross cross section and on microscopic examination no tumor was found. Gross examination of the other thoracic, lumbar and sacral vertebrae failed to demonstrate any additional tumor.

(*c*) Lungs: The right lung weighed 640 and the left 500 Gm. Both were voluminous and practically airless. Numerous infarcts of varying size and age were found in both lungs. These were associated with thrombi of corresponding variation in age in the medium and small branches of the pulmonary artery. The source of the thrombi was apparently a thrombosed left popliteal vein. No thrombi were found in the major venous trunks and their tributaries, or in the chambers of the heart or in the veins of the right leg. The vessels of the arms were not examined.

There was marked thickening of the interlobular septums. The arterial branches were likewise thickened. Microscopically, there were marked fibrous thickening of the stroma and of the arterial tree, fresh and recent infarcts and fresh and older thrombi, the latter undergoing organization. The noninfarcted areas of the parenchyma showed alveoli filled with hemosiderin-laden macrophages, precipitated protein of edema, fibrin, a few leukocytes and many red blood cells. The capillaries were markedly distended. Aside from the infarctions, the changes found corresponded well to those of pulmonary fibrosis associated with severe mitral stenosis, described by Parker and Weiss.[2]

The other findings included moderate peripheral edema of the legs, clubbing of fingers and toes and atrophy of the small muscles of the right hand. The right and left pleural cavities contained approximately 200 and 100 cc. of a clear straw-colored fluid, respectively. The abdomen contained 150 cc. of a similar fluid. Approximately 15 to 20 cc. of the same fluid was present in the pericardial sac. There were no pleural, pericardial or peritoneal adhesions. All the structures of

2. Parker, F., and Weiss, S.: Am. J. Path. **12**:329, 1936.

the superior mediastinum were displaced slightly anteriorly but not laterally by the vertebral tumor. The large deep veins of the chest and the neck were unusually distended. Examinations of the other viscera did not reveal any additional tumor sites.

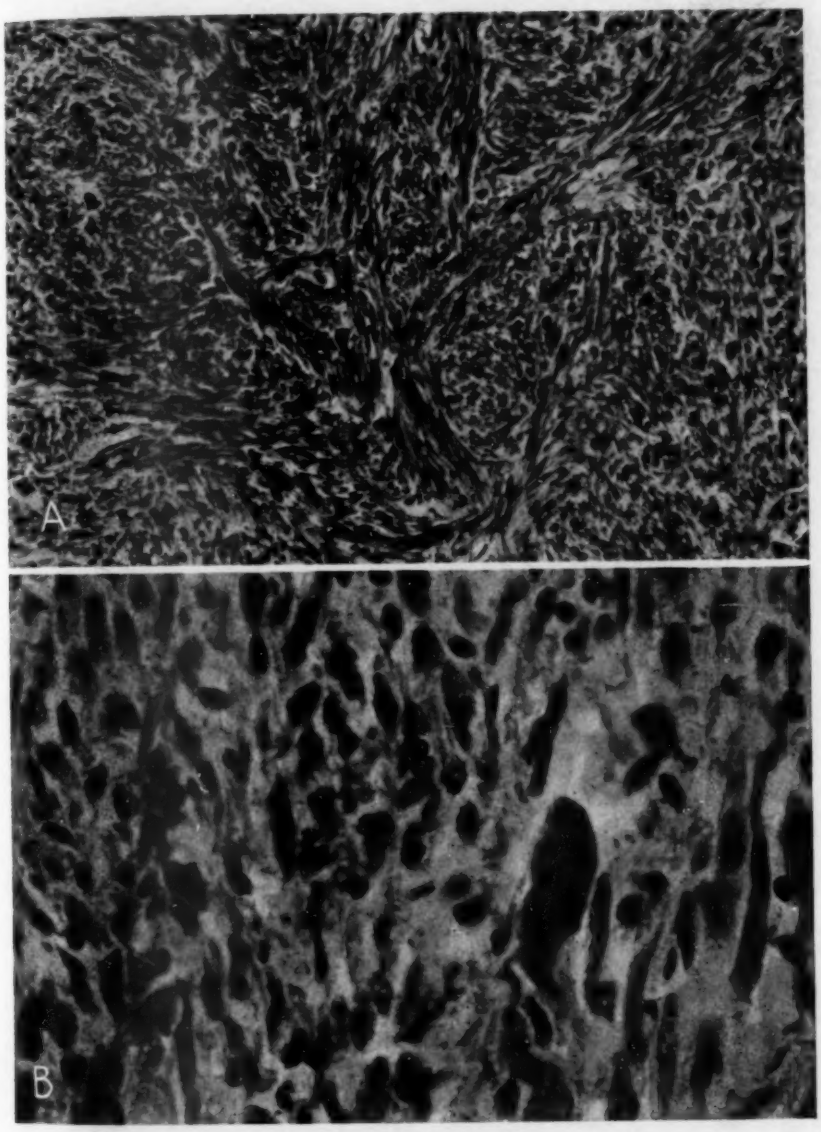

Fig. 2. *A*, microscopic view of the vertebral metastasis. The fundamental identity of pattern and cell type is well shown. (Phosphotungstic acid-hematoxylin; × 143.)

B, microscopic view of the vertebral metastasis. Tumor giant cells, fibroglia fibrils and collagen are shown. (Eosin-methylene blue; × 800.)

COMMENT

Clinical Aspects.—The antemortem diagnosis of tumor of the heart is rare. Mahaim [3] in his remarkably comprehensive and critical review of the whole subject found 23 cases in which such a diagnosis had been made. In 5 more cases the diagnosis was suspected. In 20 of the 28 cases the tumor was secondary and in 8 it was primary. Two additional cases of metastatic tumor, reported by Hsiung and associates,[4] should be added to this list. Only 3 established cases of primary intracardiac tumor recognized before death are known. These were reported by Popp [5] Barnes, Beaver and Snell [6] and Shelburne.[7] It is of interest that in none of the 30 cases was the tumor benign.

In the opinion of the observers cited, the following findings are suggestive or diagnostic of a tumor of the heart:

(1) Unexplained and intractable cardiac failure, which is often the first and the last.

(2) Unexplained and sometimes inconstant changes in cardiac rhythm, sounds and size as judged by physical, roentgen and electrocardiographic examinations.

(3) Development of a hemorrhagic pericardial effusion. (The presence of tumor cells in the fluid may confirm the diagnosis.)

(4) Unexplained signs of obstruction of the cardiac blood flow or of the blood flow of the major thoracic vessels.

(5) A specimen removed in arterial embolectomy which is shown on microscopic examination to be derived from a tumor in the heart.

In the case presented here the manifestations of the metastasis and the repeated pulmonary infarcts antedated and overshadowed the signs of the process in the heart. Yet there was progressive cardiac failure, a murmur which steadily became louder and a heart which steadily grew in size. One is tempted to speculate on the significance of the clubbing and of the patient's statement that it had been present as long as she could remember. The average duration of symptoms in most cases of tumor of the heart is under six months.[8] In this case, the presenting symptoms began approximately one year before death, while the signs of cardiac failure appeared shortly before death.

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3. Mahaim, I.: Les tumeurs et les polypes du coeur: Etude anatomoclinique, Paris, Masson & Cie, 1945.

4. Hsiung, J. C.; Szutu, C.; Hsieh, C. K., and Lieu, V. T.: Chinese M. J. **57**:1, 1940.

5. Popp, L., cited by Mahaim.[3]

6. Barnes, A. R.; Beaver, D. C., and Snell, A. M.: Am. Heart J. **9**:480, 1934.

7. Shelburne, S. A.: Ann. Int. Med. **9**:340, 1935.

8. Ritchie, G.: Am. J. Path. **17**:483, 1941.

In summary, while the diagnosis of tumor of the heart is theoretically feasible it has seldom been made. One reason for this may be the failure to consider such a possibility in the differential diagnosis. Mahaim [8] rejected the generally held view that primary cardiac tumors are great rarities. He expressed the belief that were the incidence of such tumors based not on general autopsy figures but on those of patients with cardiac disease a more nearly true picture of the situation would be obtained. According to him, the diagnosis of such tumors is not merely of academic interest. A large number of them, i. e., the auricular polyps, may be amenable to surgical treatment in the near future.

Pathologic Aspects.—The general subject of cardiac tumors was reviewed by Yater,[9] Lisa, Hirschhorn and Hart,[10] and Ritchie.[6] Primary tumors of the heart were reviewed by Haythorn, Ray and Wolff [11] and by Hamilton-Paterson and Castleden.[12] A summary of cases of primary sarcoma of the heart was made by Somolinos-D'Ardois.[13] The most comprehensive clinicopathologic survey of the whole field of cardiac tumors, including those of the pericardium, was made by Mahaim [8] in 1945. It is based on a critical review of the literature (1,298 references) and an unusually wide personal experience.

A few general conclusions of these and other observers must suffice.

Mahaim reviewed 413 cases of primary tumor of the heart—those in the literature and his own previously unreported cases. Excluded were 56 cases of organized thrombus or pseudomyxoma and a number of cases with inadequate or unreliable data. He pointed out that had the diagnosis of pseudomyxoma been based on adequate histologic study rather than on gross examination alone or on incomplete microscopic study, the total number of instances of primary tumor would undoubtedly be considerably increased. A table taken from his monograph is reproduced here because it summarizes well the classification and the distribution of the tumors.

As can be seen from the table, a classification based solely on anatomic grounds, i. e., polypoid and nonpolypoid tumors, is advanced. Despite the fact that this lumps together a wide variety of processes, the anatomic basis has diagnostic and probably therapeutic merit. The polypoid forms commonly produce signs of constant or transient interference with cardiac blood flow, and a number of the benign variants may some day be amenable to surgical removal. Thus he found 250

9. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.

10. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. **67**:91, 1941.

11. Haythorn, S. R.; Ray, W. B., and Wolff, R. A.: Am. J. Path. **17**:261, 1941.

12. Hamilton-Paterson, J. L., and Castleden, L. I.: Brit. Heart J. **4**:103, 1942.

13. Somolinos-D'Ardois, G.: Arch. latino-am. de cardiol. y hemat. **10**:1, 1940.

instances of auricular polyp. In 174 of these the growth was occlusive, and in 70 per cent of the total number it was in the left atrium.

Out of a total of 87 cases of primary sarcoma of the heart, the auricles in 61 were involved, and the right auricle in 42 of these. In at least 42 of the total the tumor metastasized, mainly to the lungs. In none did it spread to the vertebrae. Our case brings the total to 88 and, to our knowledge, is the first instance of primary tumor of the heart involving vertebrae.

Mahaim's Table of Published Cases of Primary Tumor of the Heart and the Pericardium

	Polypoid	Nonpolypoid	Total
Heart			
Myxoma	82	23	105
Fibroma	8	29	37
Lipoma	4	10	14
Angioma (including lymphangioma)	4	9	13
Rhabdomyoma	..	60	60
Celothelioma (mesothelioma) of node of Tawara	..	5	5
Miscellaneous (cysts and other benign tumors)	..	8	8
Sarcoma	21	66	87
Total	119	210	329
Pericardium			
Fibroma	7		
Lipoma	3		
Angioma	10		
Miscellaneous (cysts)	19		
Celothelioma (malignant)	24		
Sarcoma	20		
Miscellaneous (malignant)	1		
Total	84		84
			413

We believe that the tumor of the heart was primary, because metastatic tumor of the heart is multiple, involves the myocardium and is part of a widespread invasion of the body. It is unlikely that a metastasis derived from the vertebral tumor would have lodged in the left side of the heart without involvement of a lung. No cases of solitary cardiac metastasis of tumor are known (Ritchie [8]; Lisa and co-workers [10]; Burke [14]).

SUMMARY

A case of primary fibrosarcoma of the heart with a solitary metastasis, this involving the thoracic vertebrae, is presented. The clinical and pathologic aspects are briefly discussed.

14. Burke, E. M.: Am. J. Cancer **20**:33, 1934.

HISTOLOGIC CHANGES OCCURRING IN THE HEMOPOIETIC ORGANS OF ALBINO RATS AFTER SINGLE INJECTIONS OF 2-CHLOROETHYL VESICANTS

A Quantitative Study

JAMES E. KINDRED, Ph.D.
CHARLOTTESVILLE, VA.

THE PRINCIPAL object of this investigation was to assay by quantitative histologic methods the effects which intravenously injected sulfur and nitrogen mustard vesicants produced on the hemopoietic organs of the albino rat. The methods used had already been applied in a study of the hemopoietic organs of normal young adult rats.[1] Concerning sulfur mustard (mustard gas), it was known from clinical observations of victims of exposure[2] and from experimental study of laboratory animals[3] that this vesicant produces leukopenia of the circulating blood and damages the lymphoid organs, the bone marrow[3] and the intestinal mucosa.[4] During World War II, under the direction of the chemical warfare services of Canada, Great Britain and the United States, extensive experimental studies were carried on with sulfur mustard and the newly developed toxic nitrogen mustards. A summary of the results and a discussion of the concepts of the chemical, biologic and therapeutic actions of the mustards have been made by Gilman and Phillips.[5] The results described in the present paper are among those listed by name and date.

In brief, it has been shown that in laboratory animals in general the mustards caused transient hypoplasia of the lymphoid organs and

From the Anatomical Laboratory, University of Virginia.

This work was done under contract with the Medical Division of the Chemical Warfare Service.

1. Kindred, J. E.: Am. J. Anat. **71**:207, 1942.

2. (*a*) Krumbhaar, E. B., and H. D.: J. M. Research **40**:497, 1919. (*b*) Hermann, G. R.: J. Lab. & Clin. Med. **4**:1, 1918-1919.

3. (*a*) Pappenheimer, A. M., and Vance, M.: J. Exper. Med. **31**:71, 1920. (*b*) Hektoen, L., and Corper, H. J.: J. Infect. Dis. **28**:279, 1921.

4. (*a*) Lynch, V.; Smith, H. W., and Marshall, E. K., Jr.: J. Pharmacol. & Exper. Therap. **12**:265, 1918-1919. (*b*) Warthin, A. S., and Weller, C. V.: J. Lab. & Clin. Med. **4**:229 and (*c*) 265, 1919; (*d*) Winternitz, M. C.: The Pathology of War Gas Poisoning, New Haven, Conn., Yale University Press, 1920.

5. Gilman, A., and Phillips, F. S.: Science **103**:409, 1946.

of the bone marrow, accompanied by inhibition of mitosis in blast cells, which resulted in leukopenia of the circulating blood with marked initial lymphopenia, followed by neutropenia. The degenerative changes in the hemopoietic organs were accompanied by widespread systemic effects, among the most important of which were enteritis, pulmonary embarrassment, hypertrophy of the cortex of the adrenal gland and injury of the central nervous system. Certain of the nitrogen mustards produced more acute degenerative changes than others, and gradations of dose produced comparable degrees of degeneration. On the basis of the changes in the hemopoietic organs, which are notable sites of rapid cell growth, two of the agents, methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine, have been utilized in the treatment of persons suffering from neoplastic disease of the hemopoietic and other organs.[6]

The data presented in this paper are from reports presented to the Medical Division of the Chemical Warfare Service of the United States Army during 1945 and given in preliminary form before the American Association of Anatomists.[7] They include: detailed quantitative histologic analyses of the thymus, the cervical lymph nodes, the spleen and the bone marrow of rats receiving intravenous injections of sulfur and nitrogen mustards in lethal (100 per cent) doses; a quantitative study of the white blood corpuscles of the circulating blood and of the adrenal glands; a qualitative study of the lungs, the liver, the kidneys, the hypophysis, the cerebrum, the cerebellum and the intestine; gravimetric measurements of the lymphoid organs and qualitative study of these organs and of the bone marrow of adrenalectomized rats receiving injections of tris (2-chloroethyl) amine, and an in vitro study of fragments of the thymus suspended in plasma and tris (2-chloroethyl) amine.

MATERIAL AND METHODS

Male albino rats of a Wistar strain, 60 to 70 days of age and weighing between 150 and 200 Gm., were used. In the biochemical laboratory, under the direction of Dr. Alfred Chanutin, the rats were anesthetized with pentobarbital sodium and given single injections of freshly prepared solutions of sulfur mustard (bis [2-chloroethyl] sulfide) and of the hydrochlorides of the following nitrogen mustards, respectively: ethyl-bis (2-chloroethyl) amine, methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine. The sulfur mustard was dissolved in thiodiglycol (2,2′-thiodiethanol) and the nitrogen mustards in saline solution. The jugular vein was dissected free, the agent injected in approximately 0.4 cc. of solvent and the incision closed. The solvent contained about 0.5 mg. of the sulfur mustard, and 1.0 mg. of each of the nitrogen mustards, per kilogram of body weight. These doses had been found by previous assay to be lethal for the rat. Control rats

6. (*a*) Rhoads, C. P.: J. A. M. A. **131**:656, 1946. (*b*) Goodman, L. S.; Wintrobe, M. M.; Dameshek, W.; Goodman, M. J.; Gilman, A., and McLennan, M. T.: ibid. **132**:126, 1946. (*c*) Jacobson, L. O.; Spurr, C. L.; Guzman-Barron, E. S.; Smith, T.; Lushbaugh, C., and Dick, G. F.: ibid. **132**:263, 1946.

7. Kindred, J. E.: Anat. Rec. **94**:474, 1946.

were anesthetized and saline solution (0.4 cc.) alone injected into the jugular vein. All rats were starved and killed one, two, three and four days after the injection. The rats were killed by exsanguination. At the time each rat was killed, blood was drawn from the jugular vein for counts of the white blood cells and for smears. The rats that received the injection of saline solution will be referred to as the control group; those that received the injection of sulfur mustard, as the SM group; those that received the injection of ethyl-bis (2-chloroethyl) amine, as the ECA group; those that received the injection of methyl-bis (2-chloroethyl) amine, as the MCA group, and those that received the injection of tris (2-chloroethyl) amine, as the TCA group.

The thymus, the anterior and posterior cervical lymph nodes, the spleen and the adrenal glands were removed and weighed. The organs of 4 or more rats in each group were fixed in Helly's fluid, embedded in paraffin, sectioned and stained either with Delafield's hematoxylin and eosin or Feulgen's or Mallory's stain. The right femur was split, and touch-smear preparations of the marrow were made and stained by the May-Grünwald method. The proximal end of the femur and its marrow were fixed in Helly's fluid.[8] Later these femurs were decalcified in Perenyi's fluid, sectioned and stained as were the sections of the lymphoid organs. In addition, some of the sections were stained by the eosin–azure II–hematoxylin method of Maximow.

The volumes of the parts of organs were calculated by projecting images of serial sections on paper, outlining, cutting out and weighing the parts. The number of cells per unit volume was calculated by counting the cells per unit volume in one section from each organ or region of that organ under oil immersion magnification (1600 diameters). This method of calculation has been criticized by Abercrombie[9] as being subject to error when extrapolations as regards distribution per larger volume are to be made from such samples. This criticism may be valid when cells in closely packed tissues are studied, but with the experimental materials referred to here, in which the cells are rather isolated, the error is small. Furthermore, calculations of the numbers of lymphocytes produced by the lymphoid organs of the rat made by the use of this method have been found to agree with counts of lymphocytes made directly from the thoracic duct.[10]

The percentage distributions of the several types of cells observed in the thymic cortex (unit volume, 70,000 cubic microns), the anterior cervical lymph nodes (unit volume, 90,000 cubic microns) and the spleen (unit volume, 70,000 cubic microns) were obtained by counting the cells of each specific type in each of these unit volumes. In these counts degenerated cells both inside and outside macrophages were included. The cells in mitosis in 25 unit volumes from the cortices of the thymus and the anterior cervical lymph nodes and from the lymphoid cords of the spleen, respectively, and in 10 unit volumes (1 unit volume per nodule) of the secondary lymphoid nodules of one anterior cervical lymph node and of the spleen of each rat were counted.

In regard to the bone marrow, the cell populations were counted in sections (unit volume, 50,000 cubic microns), but the percentage distributions of the types of cells were obtained from differential counts of 500 cells in stained smears from the marrow of each rat. Cells in mitosis were counted in 25 unit volumes

8. Helly's fluid is a modification of Zenker's solution in which, instead of glacial acetic acid, solution of formaldehyde U. S. P. is added in the concentration of 5 per cent.

9. Abercrombie, M.: Anat. Rec. **94**:239, 1946.

10. Reinhardt, W. O.: Anat. Rec. **94**:197, 1946.

per section of marrow from each rat, and the numbers of megakaryocytes observed in these volumes were recorded.

In the spleen, in addition to the quantitative study of the lymphoid tissue, the hemopoietic areas, the cells in mitosis in these areas and the megakaryocytes per 25 unit volumes were counted.

The quantitative data with their standard errors were listed in tables which included: (1) the average number of white blood corpuscles per cubic millimeter of circulating blood; (2) differential counts of the white blood corpuscles; (3) the average numbers of lymphocytes and neutrophilic granulocytes per cubic millimeter of circulating blood, calculated from (1) and (2); (4) the average weight of the lymphoid organs per hundred grams of body weight; (5) the average volumes of parts of the lymphoid organs and bone marrow; (6) the average percentage distributions of the several different types of cells per unit volume of tissues; (7) the average number of medium-sized lymphocytes in mitosis in a specific number of unit volumes of tissue from the lymphoid organs; and the average number of myeloid and erythroid cells in mitosis in the bone marrow.

These data were extensive, and although they are presented in detail in the Chemical Warfare Service reports, it was decided in the interest of brevity to present the changes in the number of cells and of cells in mitosis in tables which summarize the results and which are based on the weight of the organ, the volume of the specific part and the numbers and percentages of the different types of cells. The extent of variability in the gravimetric measurements and in the percentage incidence of certain of the cells of the thymus, the cervical lymph nodes, the spleen and the marrow are presented in graphic form as an aid to the understanding of the general trend of changes in these organs. Furthermore, only those organs and the parts of those organs which under normal conditions contribute significantly to the lymphocyte and granulocyte populations of the blood are so presented.

Photomicrographs were made of sections of the several organs of the controls and the rats given the vesicants. Because of the similarity of the morphologic changes resulting in the organs injured by the several agents, it has been felt that the changes which occurred in the rats receiving an injection of tris (2-chloroethyl) amine would illustrate the typical conditions. Hence the photographs presented are from one day control rats and rats which had received an injection of this vesicant.

In addition to these materials, the lymphoid organs and the bone marrow of adrenalectomized rats which had received injections of varying amounts of tris (2-chloroethyl) amine were studied quantitatively and qualitatively. Starved adrenalectomized rats served as controls for this group. Also, supravital examinations were made of samples of fragments of thymus suspended in a solution of plasma and tris (2-chloroethyl) amine, the latter in a concentration equivalent to that used for intravenous injection.

EXPERIMENTAL RESULTS

THE WEIGHTS OF THE RATS

The average body weight of the control group decreased significantly on the first day after the injection (7.0 ± 0.3 per cent), was significantly lower on the second day (13.0 ± 1.0 per cent) and remained at this level throughout the fourth day. All of the vesicant-poisoned groups showed significantly less decrease in average body weights on the first day than did the control group (SM, 4.2 ± 0.8 per cent; ECA, 2.1 ± 0.6 per cent; MCA, 2.7 ± 0.4 per cent; TCA, 4.7 ± 0.9 per cent). But at the end of the second day the weight of each of these groups except

the sulfur mustard group was significantly decreased from that of the first day. The weights of the nitrogen mustard groups remained at the same level as that of the control group on the remaining days of the period, but the weight of the sulfur mustard group was not as low as that of the control group until the fourth day. These data indicate that any losses of weight suffered by the rats given the vesicants were caused by acute inanition and not by the agents.

THE WHITE BLOOD CELLS OF THE CIRCULATING BLOOD

There were no significant changes of the average number of white blood cells of the circulating blood of the controls throughout the four day period (table 1, A). In all of the rats that received vesicants there was leukopenia initially. The average

TABLE 1.—*White Blood Corpuscles*

Rats were given single intravenous injections of saline solution, sulfur mustard (SM), ethyl-bis (2-chloroethyl) amine (ECA), methyl-bis (2-chloroethyl) amine (MCA) and tris (2-chloroethyl) amine (TCA), respectively. The average numbers of white blood corpuscles, lymphocytes and neutrophilic granulocytes per cubic millimeter of blood are recorded as found one, two, three and four days after the injection, respectively. WBC. means white blood corpuscles; L., lymphocytes; P., neutrophilic granulocytes; No., number of rats; v., standard error of mean.

A. Average Numbers of White Blood Corpuscles per Cubic Millimeter

	Saline Solution			SM			ECA			MCA			TCA		
Days	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.
1	19	5,400	± 355	6	2,400	± 142*	6	2,100	± 500*	13	2,100	± 100*	6	1,830	± 246*
2	8	5,400	± 690	6	1,300	± 180†	10	1,125	± 165	10	1,150	± 200†	16	1,100	± 187†
3	14	4,950	± 507	6	3,600	± 1,010	4	1,800	± 500	8	850	± 118	6	1,000	± 158
4	13	6,400	± 510	7	4,400	± 800	.....			10	650	± 58	7	1,075	± 240†

B. Average Numbers of Lymphocytes per Cubic Millimeter

	No.	L.	v.	No.	L.	v.	No.	L.	v.	No.	L.	v.	No.	L.	v.
1	19	4,150	± 300	6	1,720	± 210*	6	1,110	± 420*	13	1,110	± 160*	6	455	± 84*
2	8	3,900	± 525	6	730	± 150†	10	310	± 270	10	630	± 167†	16	177	± 40†
3	14	3,400	± 390	6	1,500	± 750	4	325	± 62	8	690	± 108	6	520	± 115†
4	13	4,600	± 670	7	880	± 580	.....			10	475	± 40	7	570	± 120

C. Average Numbers of Neutrophilic Granulocytes per Cubic Millimeter

	No.	P.	v.	No.	P.	v.	No.	P.	v.	No.	P.	v.	No.	P.	v.
1	19	1,070	± 310	6	650	± 168	6	950	± 130	13	950	± 85	6	1,350	± 176
2	8	1,350	± 220	6	550	± 68	10	815	± 137	10	500	± 134†	16	920	± 152
3	13	1,430	± 500	6	2,000	± 700†	4	1,420	± 1,000	8	138	± 43†	6	450	± 270
4	13	1,600	± 150	7	3,450	± 500	.....			10	155	± 30	8	1,040	± 83†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

count was reduced to 45 per cent of the control value in the SM group, to 39 per cent in the ECA and MCA groups and to 33 per cent in the TCA group. The leukopenia was more severe on the second day. On the third day, the control level was reached in the SM group, but the leukopenia continued throughout the remainder of the period in the nitrogen mustard groups. There appeared to be a slight alleviation of the leukopenia in the TCA group, but not in the MCA group, on the fourth day. No members of the ECA group survived beyond the third day.

The percentages of the several types of white blood cells were determined from differential counts of 100 cells of one smear of the blood of each animal (table 2). Only this number was counted because the distribution was so sparse in rats in which the total white blood cell count was less than 1,000 per cubic millimeter.

There were no significant changes of the percentage distributions of the myelocytes in any of the groups throughout the period. The juvenile neutrophils were

quite variable in their distribution but were most consistent in distribution in the control group. They decreased significantly in the MCA and TCA groups throughout the period. In the SM group they were significantly less numerous than in the control group until the fourth day.

The band neutrophils were practically absent from the blood of the controls throughout the period. In the poisoned rats their distribution was variable, and the only significant changes noted in a comparison of these and the control rats were increases in the TCA group on the first and fourth days and in the SM group on the third and fourth days. Thus the only groups to show a shift to the left in the neutrophil distribution at some time during the period were the TCA and SM groups.

TABLE 2.—*White Blood Corpuscles*

Rats were given single intravenous injections of saline solution and vesicants as listed, respectively. The average percentage distributions of juvenile (J-N), band (B-N) and segmented (S-N) neutrophilic granulocytes, lymphocytes (L.) and eosinophilic granulocytes (Eo.) are recorded as found in blood smears one, two, three and four days after injection, respectively; 100 cells were counted in one smear from each rat. Neg. means distribution less than 0.1 per cent; other abbreviations have the meanings given in table 1. Myeloblasts, myelocytes and monocytes are not included.

	No.	J-N. v.	B-N. v.	S-N. v.	L. v.	Eo. v.
A. One Day After Injection						
Saline	13	3.4 ± 1.0	Neg.	16.5 ± 2.0	76.0 ± 2.5	1.4 ± 0.3
SM	6	0.5 ± 0.2*	0.5 ± 0.2*	26.0 ± 3.0	72.0 ± 3.0	Neg.*
ECA	6	3.0 ± 0.8	1.0 ± 0.8	41.0 ± 8.0*	53.0 ± 9.5*	0*
MCA	6	2.0 ± 0.7	1.3 ± 0.7	42.0 ± 5.0*	53.0 ± 4.5*	0.6 ± 0.1
TCA	9	1.0 ± 0.5*	4.0 ± 0.5*	69.0 ± 7.7*	25.0 ± 6.0*	0*
B. Two Days After Injection						
Saline	8	2.3 ± 0.5	0.6 ± 0.3	22.2 ± 3.7	72.0 ± 3.6	1.0 ± 0.5
SM	6	0.5 ± 0.2*	0.8 ± 0.4	41.0 ± 4.2*	57.0 ± 4.6*	0.5 ± 0.3
ECA	8	1.0 ± 1.0	1.0 ± 0.3	70.0 ± 7.5*	27.0 ± 7.0*	0
MCA	7	0.5 ± 0.3*	0.5 ± 0.2	43.0 ± 8.4	55.0 ± 8.0	1.0 ± 0.3
TCA	15	Neg.*	1.0 ± 0.3	82.0 ± 2.9*	16.0 ± 2.6*	Neg.
C. Three Days After Injection						
Saline	8	4.0 ± 1.3	Neg.	25.0 ± 1.8	68.0 ± 2.4	1.0 ± 0.3
SM	6	1.7 ± 0.7*	3.5 ± 1.1*	50.0 ± 6.3*	42.0 ± 6.7*	Neg.
ECA	4	6.0 ± 2.4	4.0 ± 2.6*	71.0 ± 2.8*	18.0 ± 2.6*	0.2 ± 0.3
MCA	10	0.6 ± 0.2*	0.7 ± 0.4	15.0 ± 4.9	82.0 ± 4.5	Neg.
TCA	6	3.0 ± 0.9	2.0 ± 0.4*	40.0 ± 3.4*	52.0 ± 3.0*	Neg.
D. Four Days After Injection						
Saline	6	5.0 ± 0.4	Neg.	20.0 ± 1.9	72.0 ± 3.0	1.3 ± 0.3
SM	7	3.6 ± 0.6	5.4 ± 2.6	69.0 ± 7.0*	20.0 ± 6.4*	0*
ECA	..	.....	.....	.....	.....	.....
MCA	10	2.0 ± 0.4*	2.0 ± 0.8*	20.0 ± 3.7	73.0 ± 4.5	0*
TCA	11	1.5 ± 0.2*	3.3 ± 1.9*	58.0 ± 3.7*	34.0 ± 3.7*	0.3 ± 0.6

* The number is significantly different from the mean of the controls (saline solution).

The segmented or mature neutrophils showed no significant changes of distribution in the control group. There was initial relative increase of these cells in the nitrogen mustard groups. This initial increase persisted in the ECA and TCA groups but returned to the control level in the MCA group. The SM group did not show significant change until the second day, but the percentage continued to rise until the fourth day.

There were no significant changes of the percentage distributions of the lymphocytes throughout the period in the control group. There were initial significant decreases of percentage distributions of these cells in the nitrogen mustard groups. In the ECA and TCA groups these decreases continued throughout the period in the surviving rats (Note: there were no rats which survived the injection of ethyl-bis (2-chloroethyl) amine beyond the end of the third day.) In the MCA

group the percentage returned to the control level at the end of the second day and so continued until the end of the fourth day. In the SM group the only significant change throughout the period was a significant decrease at the end of the third day. The ECA and TCA groups varied the most consistently from the control group.

The eosinophilic granulocytes were distributed in very small numbers in the controls but were always present, whereas these cells were practically never present in the poisoned rats throughout the period. There were no significant changes in the incidence of the monocytes throughout the period in any of the groups, and basophilic granulocytes were so few that no changes could be observed.

There were no significant differences between the numbers of degenerated cells observed in the blood smears of the control rats and those of the poisoned rats.

In order to evaluate the trend of change in the absolute numbers of lymphocytes and neutrophilic granulocytes, the basic data of tables 1 A and table 2 were combined, and the results of these calculations are presented in table 1 B and C. There were no significant changes of the average number of lymphocytes per cubic millimeter in the control group throughout the period (table 1 B and fig. 1). In the poisoned groups there was initially marked lymphopenia. The relative decreases in the several groups in terms of the average of the first day control rats are shown in table 5. This lymphopenia was relatively greater than the leukopenia in all but the SM group. On the second day the lymphopenia became more severe in all groups. On the third day it remained at the lowest levels reached in the ECA and MCA groups, but the lymphocytes increased in number in the TCA and SM groups. On the fourth day there were significant decreases in the numbers of lymphocytes in the MCA and SM groups, but there was no change in the TCA group. Thus the counts of lymphocytes of the blood showed decreases proportionately greater than the decreases of the total leukocyte counts, and the initial leukopenia and lymphopenia were greatest in the TCA group and least in the SM group. The ECA group showed the greatest decrease after the first day, the MCA group showed no indication of regeneration of lymphocytes, while in the TCA and SM groups this was initiated on the third day, but showed relapse in the SM group on the fourth day. The amelioration of the leukopenia by increase of lymphocytes was characteristic of the TCA and SM groups.

In the control group there were no significant changes of the neutrophil count throughout the period (table 1 C and fig. 10). In the poisoned groups there were no significant changes of the neutrophil counts on the first day. On the second day the only group to show significant change was the MCA group, in which there was significant neutropenia. On the third day there was significant neutrophilia in the ECA and SM groups (70 and 260 per cent neutrophils, respectively), which was paralleled in the SM group by significant increase in the total leukocyte count. The MCA and TCA groups showed significant decreases in the neutrophils of 72 and 51 per cent, respectively. In the MCA group this decrease resulted in continued decrease of the total leukocyte count, but in the TCA group this decrease was neutralized by marked increase in the number of lymphocytes, so that the total leukocyte count did not change. On the fourth day, the neutrophils remained at the low level of the third day in the MCA group, but their number increased in the TCA and SM groups by 130 and 50 per cent, respectively. In these groups the rises accounted for the increases in the total leukocyte counts of the fourth day.

In brief, the data show that the number of neutrophils of the circulating blood was not changed in the ECA group but was significantly reduced in the MCA and in the TCA group; in the TCA group, however, it showed recovery. In the SM group there was neutrophilia on the third and fourth days. In contrast with the

neutrophils, the lymphocytes were markedly reduced in number, so that the leukopenia observed in the SM, ECA and TCA groups was caused by the lymphopenia, and that in the MCA group by both lymphopenia and neutropenia, with the lymphopenia having the greatest influence on the total leukocyte count.

THE THYMUS

The average relative weight of the thymus fluctuated in the control group throughout the period (fig. 1). It was significantly lower at the end of the second and fourth days than at the end of the first day. Despite these fluctuations, the average relative thymic weights of the nitrogen mustard groups were significantly less than that of the control group at all times. The average thymic weight of the sulfur mustard group was not less than that of the control group until the end of the third and fourth days, and at the end of the fourth day the value was the lowest reached in any of the groups. In the MCA and TCA groups the weight decreased throughout the end of the third day and remained at the lowest level through the fourth day. The relative decreases in the average thymic weights of the poisoned groups as compared with the average of the control group at the end of the first day are shown in table 5.

There were no significant changes of the average relative volume of the cortex of the thymus in the control group during the first three days after injection (fig. 1), but at the end of the fourth day it was significantly smaller than on the preceding days (85 per cent of the volume of the first day). Among the poisoned groups the volumes were significantly less in the nitrogen mustard groups than in the control group at the end of the first day (ECA and MCA, 86 per cent of the control; TCA, 65 per cent). In the MCA and TCA groups the volumes remained less than the control throughout the four day period. In the ECA group the volume was variable and showed return to control level at the end of the second day, but was less than the control on the third day. In the SM group there was no decrease in relative volume until the end of the second day (70 per cent of the control), and it remained at the lower level throughout the period.

The combination of dense cortex and light medulla with sharp demarcation between these parts as seen under low magnification, a condition which is characteristic of the normal thymus of the rat of this age, was present in the thymuses of the controls throughout the period. Figure 2 *a* shows the characteristic morphologic aspect of the thymic lobules as seen in the controls. In all of the poisoned rats there were no gross alterations of this pattern on the first day (fig. 2 *b*), but in the cortices of all there were light spots ("pits") where macrophages were active in areas of cell degeneration (fig. 3 *b*). On the second day, only in the SM group was the pattern unchanged; in the ECA and MCA groups, the cortex was thin and loose, and there was only a faint irregular line of demarcation between cortex and medulla; in the TCA group, there was no line of demarcation between these regions, and small lymphocytes were scattered irregularly through both (figs. 2 *c* and 3 *c*). On the third day there was no line of demarcation between cortex and medulla in any of the nitrogen mustard groups (figs. 2 *d* and 3 *d*). There were more small lymphocytes in the medulla than in the cortex. On the fourth day the same pattern was characteristic of all groups, including the SM group (figs. 2 *e* and 3 *e*).

The cortex of the thymus is composed of a reticular framework in which are found large, medium-sized and small lymphocytes, reticulum cells and a few macrophages. A blood capillary plexus and a lymphatic network are also present, ramifying between the cells. The medium-sized lymphocytes are active mitotically,

and it is believed that they form an important center of the production of lymphocytes for the circulating blood of the rat.[1] Normally there is little cell degeneration. Other investigators have observed that following treatment with the vesicants there occurred destruction of lymphocytes, decrease of mitotic cells and shrinkage of the cortex.[5] No quantitative study has been made by these investigators. The present study is an attempt to measure these changes in the

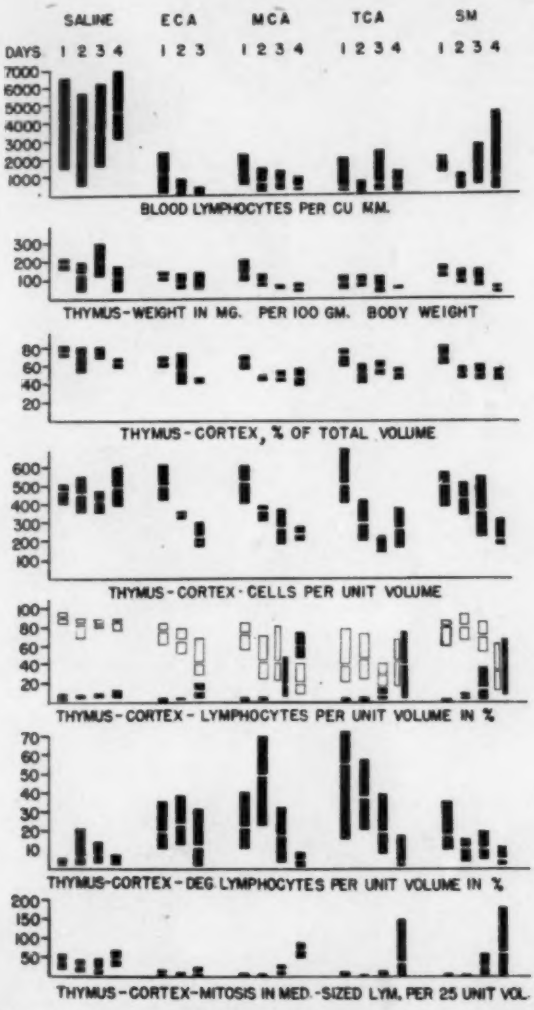

Fig. 1.—Block diagrams showing distributions of the lymphocytes of the circulating blood, the weight of the thymus, the volume of the thymic cortex, and distributions of the lymphocytes of the cortex of rats one to four days after single injections of saline solution and nitrogen and sulfur mustards. A white line across a black column indicates the mean, the upper end of the column the maximum and the lower end the minimum value, except in the first section on the lymphocytes of the thymic cortex, where the black columns represent distributions of the medium-sized lymphocytes, and the white columns those of the small lymphocytes. In the white columns the space across the column is the mean.

thymuses of vesicant-poisoned rats. It was thought that these quantitative changes could be demonstrated most favorably by extrapolation, the raw data on the relative weight of the thymus, the relative volume of the cortex and the incidence of the cells and of the cells in mitosis as they occurred in volumes of specific size being

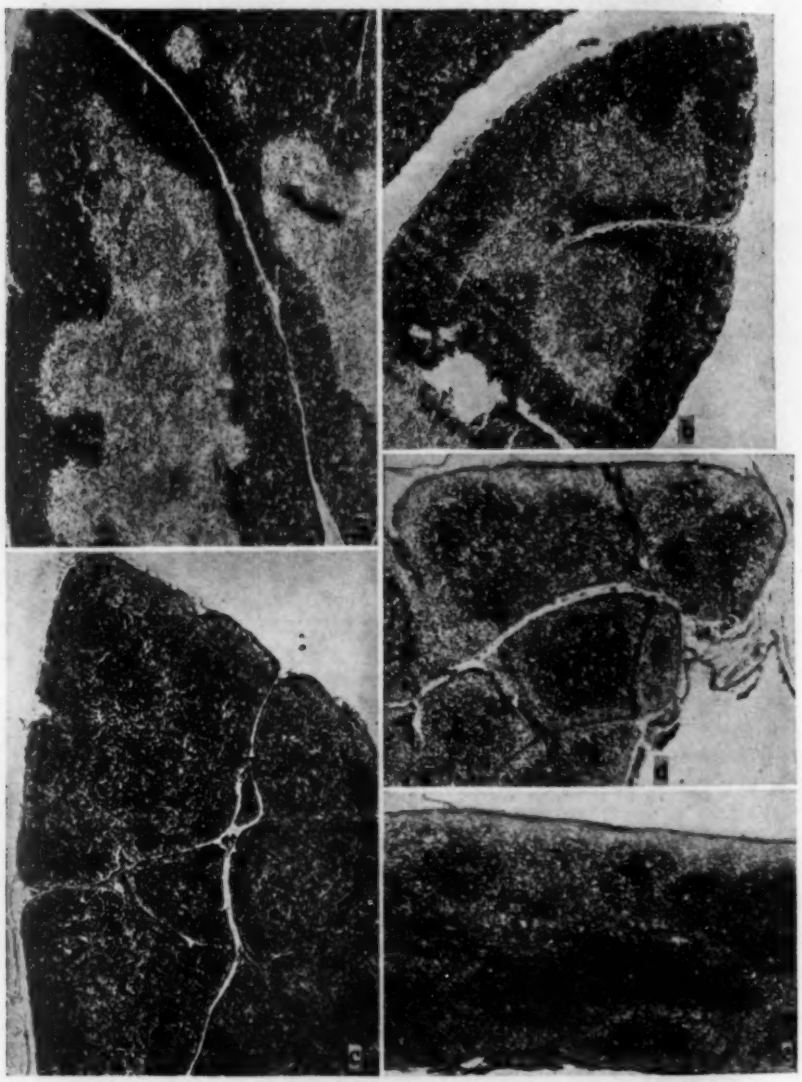

Fig. 2.—Photomicrographs of lobules of the thymus; hematoxylin and eosin; sections 7 microns thick; × 43. (*a*) Control one day after injection of saline solution. (*b, c, d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

used (fig. 1). It is acknowledged that this method is not mathematically absolute, but it is felt that the results obtained by its use give a more objective view of the morphologic changes than would description based on qualitative findings alone.

All of the raw data on cell distributions are not included in this paper, because it was thought that the larger view presented by extrapolation would be sufficient to show the trends of change. From the combined data on weight of thymus, volume of cortex and incidence of cells and of cells in mitosis (fig. 1), the data in table 3 have been calculated. These data show the percentage distributions of the

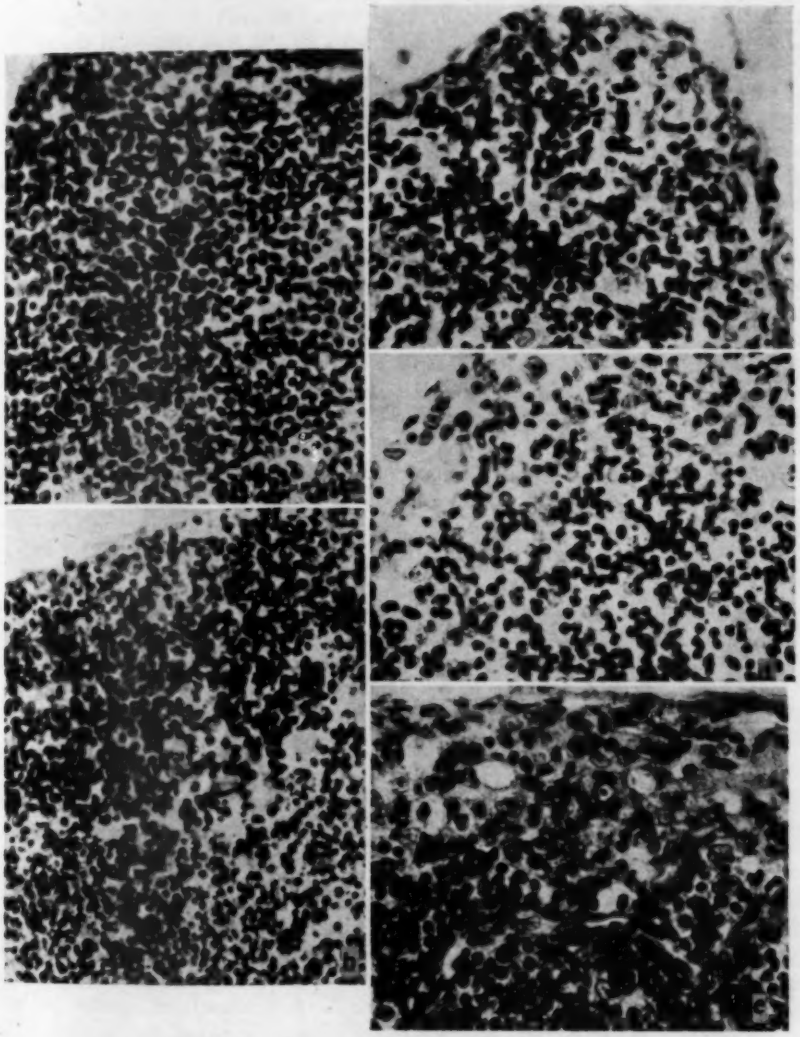

Fig. 3.—Photomicrographs of thymic cortex from sections shown in figure 2; × 201.5. (*a*) Control one day after injection of saline solution. (*b*, *c*, *d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

several types of cells and the incidence of mitosis in the cells in the thymic cortex per hundred grams of body weight on four successive days following the injection of saline solution and of the vesicants. Any value which is distinguished by an

asterisk deviated significantly from the control value of the first day, and any value which is marked by a dagger is significantly different from the value of the preceding day in the group.

In the control group there were fluctuations in the total cell population of the thymus and a tendency toward reduction in number, so that on the fourth day the population was significantly less than it was on the first day (table 3 A). This reduction is believed to reflect the effects of acute inanition. In all of the poisoned groups there was significant hypoplasia of cells on the first day. On the second day the hypoplasia was more severe, and it continued in severity on the third day in all except the SM group. On the fourth day there were no significant changes from the third day except in the SM group, in which there was a marked decrease.

In the control group the large and the small lymphocytes of the thymus were significantly reduced in number as a result of inanition (table 3 B and D). However, the reductions which occurred in the controls were negligible when contrasted with the hypoplasia of all types of lymphocytes which took place on the first day in all of the poisoned groups. The large lymphocytes never recovered from the initial hypoplasia in any of the poisoned groups, although there was a tendency toward regeneration on the third day in the SM and MCA groups and on the fourth day in the TCA group. The medium-sized lymphocytes began to regenerate in the MCA group on the third day, and there was a trend toward regeneration in the SM group (table 3 C). In all of the poisoned groups the numbers of medium-sized lymphocytes were significantly greater on the fourth day than on the first.

The initial hypoplasia of the small lymphocytes was not relatively as great in any of the groups as were the reductions in the numbers of the larger lymphocytes, but in contrast with the tendency toward regeneration of the larger forms, the small lymphocytes continued to decrease throughout the four day period (table 3 D). Tris (2-chloroethyl) amine seemed to have the greatest hypoplastic effect on the small lymphocytes.

Coincident with the decrease in the numbers of medium-sized lymphocytes there was initial significant decrease in the numbers of medium-sized lymphocytes in mitosis (table 3 F). This was greatest in the TCA group and least in the MCA group. However, the decrease in the numbers of cells in mitosis was relatively greater than the decrease in the numbers of medium-sized lymphocytes; hence it is believed that the vesicants not only destroy lymphocytes but inhibit mitosis. This inhibition increased in severity in the TCA and MCA groups on the second day but did not change in the other groups. It seemed to have ceased in the MCA and in some members of the SM group on the third day, and there was evidence of resumption of mitotic activity. This occurred in the TCA group on the fourth day. The SM group showed the greatest variation of any of the groups. It is believed that the tendency for increase in the number of medium-sized lymphocytes and in the number of these cells in mitosis is an indication of a trend toward regeneration of the thymus and renewed production of lymphocytes destined to be used by the blood.

Degenerated lymphocytes were few, variable in number and usually isolated in the controls (table 3 E and fig. 1). In the nitrogen mustard groups there were initially significantly more degenerated lymphocytes than in the control group, but in the sulfur mustard group, although in some of the rats the thymus contained large numbers of degenerated lymphocytes, there was no uniform extensive degeneration such as occurred in the nitrogen mustard groups. These degenerated lymphocytes were largely included within macrophages of reticulum cell origin and were more numerous at the margin of the cortex than in the deeper regions. Also they tended to accumulate around blood vessels as if the reticulum cells which

ingested them had arisen from modification of periarterial reticulum cells. In the nuclei of these degenerated cells the chromatin was broken up into small pyknotic droplets which gave a positive reaction for desoxyribonucleic acid in Feulgen-stained preparations.

TABLE 3.—*The Thymus*

Rats received single injections of saline solution and vesicants as listed, respectively. The average numbers of cells of different types ($\times 10^4$) and of medium-sized lymphocytes in mitosis ($\times 10^2$) in the cortex per hundred grams of body weight are recorded as found one, two, three and four days after injection, respectively. The number of organs used per day were: saline control, 6; SM, 5, 5, 4, 4; ECA, 6, 6, 4; MCA, 6, 5, 6, 9; TCA, 6. The distributions of cells were calculated from the data in figure 1 and more detailed unlisted data on large lymphocytes, reticulum cells and macrophages. Each average is followed by its standard error.

Days	Saline Solution	SM	ECA	MCA	TCA
		A. Cells of All Types			
1	94,600 ± 5,850	57,000 ± 8,600*	59,000 ± 4,500*	58,700 ± 7,600	56,900 ± 12,300*
2	60,000 ± 13,800	30,000 ± 2,800†	24,500 ± 3,140†	21,000 ± 3,460†	20,000 ± 3,900†
3	77,000 ± 8,550	28,000 ± 10,300	7,400 ± 2,280†	10,200 ± 935†	6,500 ± 1,070†
4	58,000 ± 10,300	7,000 ± 1,680	.....	9,300 ± 850	11,200 ± 1,680
		B. Large Lymphocytes			
1	460 ± 45	35 ± 18*	90 ± 34*	122 ± 48	16 ± 16*
2	400 ± 137	80 ± 29	113 ± 61	33 ± 19†	50 ± 28
3	570 ± 112	143 ± 70	29 ± 4	156 ± 53†	8 ± 8
4	200 ± 61†	26 ± 26†	.....	116 ± 27	131 ± 48†
		C. Medium-Sized Lymphocytes			
1	5,000 ± 770	800 ± 375*	670 ± 314*	655 ± 221*	633 ± 238*
2	3,520 ± 1,000	910 ± 351	562 ± 116	360 ± 123	335 ± 100
3	4,800 ± 530	3,050 ± 1,280	774 ± 165	2,450 ± 1,000†	473 ± 153
4	3,870 ± 730	2,345 ± 1,230	.....	6,000 ± 640†	4,210 ± 1,520†
		D. Small Lymphocytes			
1	82,000 ± 6,000	45,000 ± 8,452*	43,000 ± 2,700*	44,000 ± 8,000*	19,400 ± 4,500*
2	51,500 ± 12,300	24,000 ± 3,015	14,700 ± 2,120†	8,213 ± 2,130†	9,800 ± 4,000
3	62,800 ± 6,200	20,000 ± 8,000	2,930 ± 650†	3,600 ± 1,150†	1,180 ± 1,300†
4	51,200 ± 9,100	3,100 ± 1,000†	.....	1,730 ± 320†	5,000 ± 1,520†
		E. Degenerated Lymphocytes			
1	2,300 ± 480	9,000 ± 2,680*	12,600 ± 3,560*	11,500 ± 1,530*	34,100 ± 11,200*
2	2,850 ± 585	3,000 ± 545†	5,800 ± 1,740	10,600 ± 3,000	6,775 ± 1,070†
3	6,030 ± 1,820	2,700 ± 880	707 ± 310†	1,911 ± 315†	1,250 ± 342†
4	1,670 ± 555	281 ± 120†	.....	255 ± 62†	400 ± 183†
		F. Medium-Sized Lymphocytes in Mitosis			
1	3,340 ± 620	150 ± 62*	250 ± 125*	800 ± 28*	66 ± 66*
2	2,120 ± 460	70 ± 24	100 ± 32	33 ± 16†	13 ± 8
3	1,620 ± 490	550 ± 410	150 ± 60	218 ± 83†	44 ± 27
4	2,200 ± 460	855 ± 675	.....	1,370 ± 351†	1,140 ± 600
		G. Reticulum Cells			
1	2,460 ± 270	950 ± 315*	1,100 ± 235*	964 ± 296*	600 ± 282*
2	2,850 ± 585	1,651 ± 615	2,756 ± 710	1,150 ± 350	2,710 ± 635†
3	1,570 ± 215	800 ± 129	2,840 ± 1,320	1,870 ± 730	1,776 ± 445
4	1,300 ± 314	1,000 ± 310	.....	1,188 ± 128	1,170 ± 130
		H. Macrophages			
1	50 ± 25	930 ± 235*	900 ± 710*	1,060 ± 167*	2,156 ± 600*
2	180 ± 72	140 ± 31†	506 ± 100	100 ± 40†	423 ± 240†
3	80 ± 36	500 ± 71†	170 ± 61†	150 ± 66	1,100 ± 258
4	170 ± 74	102 ± 16†	.....	250 ± 60	280 ± 107†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

When contrasts were made between the numbers of degenerated lymphocytes and the reductions of the lymphocyte populations as a whole in the poisoned rats, it was found that only a small percentage of the loss of lymphocytes could be accounted for by the degeneration. Hence it is believed that the greater part of the loss of lymphocytes was caused by migration of lymphocytes. This activity would

be related directly to the inhibition of mitosis of medium-sized lymphocytes and the consequent loss of lymphocytes which normally would enter the circulation. The unaffected lymphocytes would migrate to the blood stream in an attempt to alleviate the effects of decreased production. This view was partly substantiated by the observation that when regeneration began in the thymus there was a certain degree of alleviation of the lymphopenia of the blood. This correlation was not so great as the correlation between the destruction of medium-sized lymphocytes and the inhibition of their mitosis and the initial lymphopenia of the blood.

After the initial increase of the numbers of degenerated thymic lymphocytes of the poisoned groups there was significant decrease of the numbers of degenerated cells on the second day in all except the MCA group. Methyl-bis (2-chloroethyl) amine had apparently a more lingering toxic effect than did the other agents. After the second day the numbers of degenerated lymphocytes of all groups were negligible. Since the numbers of small lymphocytes decreased at this time, it is evident that these lymphocytes were not lost in the thymus by degeneration. As pointed out in the foregoing paragraph, it is more probable that the majority migrated and that those which remained grew into medium-sized lymphocytes, thus accounting for the increase in the numbers of these forms on the fourth day.

Normally, the reticulum cells of the thymic cortex show little phagocytic activity, and it can be seen at once that the macrophages of reticulum cell origin in the control group are negligible in distribution (table 3 H). However, in all of the poisoned groups there were marked initial significant hyperplasia of the macrophages and significant hypoplasia of the unmodified reticulum cells (table 3 G). The macrophages contained degenerated lymphocytes. Beginning on the second day, there were significant reductions in the macrophage populations and a trend toward restoration of the numbers of unmodified reticulum cells. No doubt some of these macrophages perished as a result of their unusual phagocytic activity, but the majority apparently became inactive after having digested the degenerated lymphocytes and appeared morphologically as unmodified reticulum cells. The reticulum cells were apparently not injured directly by the agents, and such loss of reticulum cells as occurred was secondary to their reaction to the unusual function of phagocytosis.

It is interesting to note that as the lymphocytes were being digested by the macrophages the chromatin of the nuclei of the ingested cells retained its specific reaction to Feulgen's reagent, because of the presence of desoxyribonucleic acid. After digestion there were no traces of desoxyribonucleic acid in the cytoplasm of the reticulum cells. Since the number of degenerated lymphocytes within macrophages decreased markedly in all groups except the MCA group on the second day, it is believed that this decrease may be used as evidence for the conclusion that it takes twenty-four hours for a macrophage to digest an injured lymphocyte. This view of course is based on circumstantial evidence, but it coincides with the observation of Clark and Clark [11] on the time required for a macrophage to digest a nucleated erythroid cell in the tadpole's tail. I have used this time relation in calculating the production-destruction cycle of the lymphocytes in the lymphoid organs of normal rats.[1]

In brief, the quantitative data presented appear to indicate that the vesicants produce marked histologic changes in the thymus as a result of their destructive action on the larger lymphocytes and their inhibitive action on the mitotic mechanism of the medium-sized lymphocytes. The reticulum cells are not injured directly by the agents, but many react as macrophages and ingest and digest the

11. Clark, E. R., and Clark, E. L.: Am. J. Anat. **46**:91, 1930.

degenerated lymphocytes. Some of the reticulum cells perish as a result of this activity. The toxic effects of the agents apparently last for two days, after which there is regeneration of the larger lymphocytes and resumption of mitosis. The decrease in the weight of the thymus is thought to be largely due not to the degeneration of the cells but to migration of small lymphocytes. These lymphocytes which normally offer a large margin of safety in the supply-demand relation between the thymus and the circulating blood are drained from the thymus when the normal proliferative supply is cut off and thereby prevent total lymphopenia of the blood during the height of the toxic action of the agents.

THE CERVICAL LYMPH NODES

The average relative weight of the cervical lymph nodes was significantly lower on the second than on the first day in the control group, but on the third and fourth days there were no significant differences from the weight of the first day (fig. 4). It appears that acute inanition had a temporary depressing effect on these nodes. Among the vesicant-poisoned groups there was no initial change in the relative weight of the lymph nodes in the ECA group, but in the other groups the weights were significantly lower than that of the control (table 5). On the second day the weights in all of the vesicant-injured groups were significantly lower than that in the control group; furthermore, in the ECA and MCA groups the weights were lower than on the first day in these groups; in the TCA group the weight was significantly higher, and there was no change in the SM group (fig. 4). There were no further significant changes in the ECA, MCA and SM groups throughout the period, but in the TCA group there was a significant decrease on the third day which continued into the fourth day (table 5). In brief, there were significant decreases in the weights of the cervical lymph nodes in all groups after the first day.

Normally the cortex of the lymph node outside of the nodules produces only enough lymphocytes to maintain local growth and replace degenerated cells.[1] There is normally little degeneration, and the lymphocytes, predominantly of the small variety, are closely packed into the reticular stroma along with a small number of larger lymphocytes and reticulum cells. Cells in mitosis are few. These conditions were present in the cervical lymph nodes of the controls (fig. 5 *a*). Comparative quantitative study of the relative volumes of the cortices, the numbers of cells per unit volume, the distributions of the different types of cells and the numbers of cells in mitosis showed that in the samples from the poisoned groups the volume of the cortex shrank in proportion to the shrinkage of the whole node (fig. 5 *b* to *e*). There were marked hypoplasia of the lymphocytes, marked increase in degenerated lymphocytes, particularly around the periphery, and marked initial increase in macrophages which ingested degenerated lymphocytes. There was no generalized necrosis, and little fibrosis; the latter when present was near the hilus. The loss of lymphocytes could not be accounted for by the number of degenerated lymphocytes. It is believed that the great majority of lymphocytes which were lost from the lymph nodes entered the blood stream and with the lymphocytes from the thymus prevented complete lymphopenia of the circulating blood. After three days the degenerated lymphocytes had practically disappeared from the cortex and there were indications of regeneration and maintenance of the lymphoid character, although the nodes were smaller than normal. This part of the node is viewed as an accessory that prevents lymphopenia when the normal sources of the lymphocytes of the circulating blood are cut off. The quantitative data used as bases for the foregoing description are on file in this laboratory and in the files of the Medical Division of the Chemical War Service.

There was no significant change in the average relative volume of the secondary lymphoid nodules of samples from the anterior cervical lymph nodes of the control group throughout the period (fig. 4). In the poisoned groups there were no significant differences from the control group until the second day. At this time in the ECA, TCA and SM groups the relative volumes of the nodules were significantly lower than that of the control group. In the MCA group the relative volume did not change throughout the period. In the TCA group the relative vol-

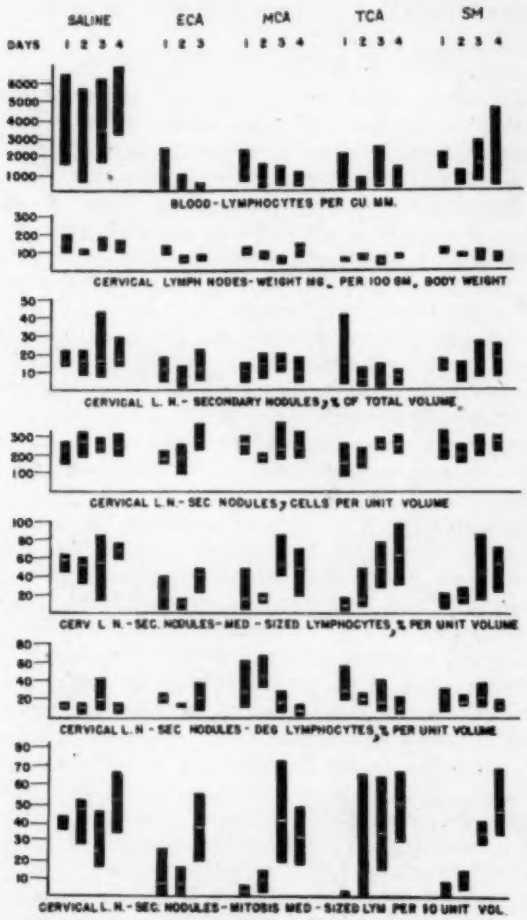

Fig. 4.—Block diagrams showing distributions of the lymphocytes of the circulating blood, the weight of the lymph nodes, the volume of the nodules and the numbers of cells of the nodules of the anterior cervical lymph nodes of rats one to four days after single injections of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

ume of the nodules remained lower than that of the control group throughout the remainder of the period, but in the ECA and SM groups the relative volumes reached the control level on the third day. Thus, only in the TCA group was the relative volume of the nodules markedly reduced. But it must be remembered

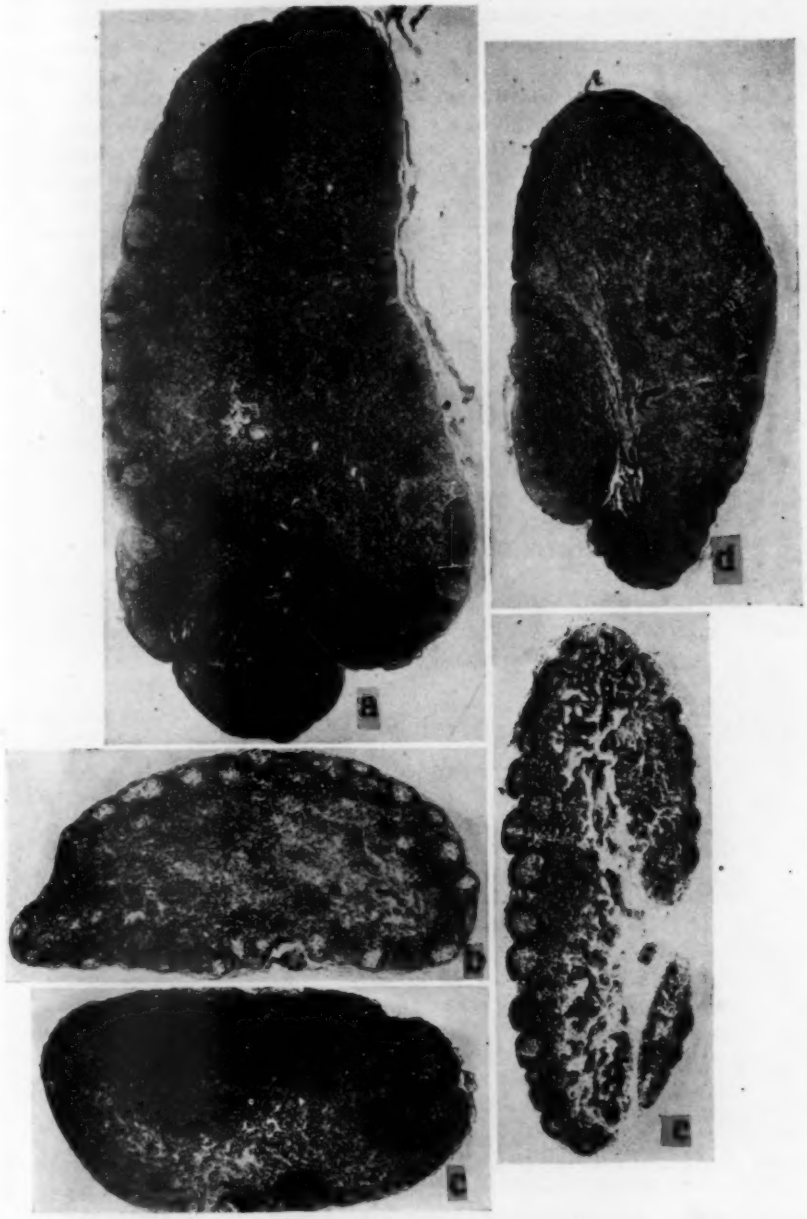

Fig. 5.—Photomicrographs of median vertical sections of anterior cervical lymph nodes; hematoxylin and eosin; sections 9 microns thick; × 16. (*a*) Control one day after injection of saline solution. (*b, c, d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

that although the relative volumes of the nodules did not change much, the volumes of the whole nodes were significantly reduced by the action of the agents, so that actually there was significant reduction in the amount of lymphoid tissue in the nodules as a result of the action of the agents (figs. 5 and 6).

The secondary lymphoid nodule of the anterior cervical lymph node of the normal rat is a large ovoid structure which is roughly divided into two parts when viewed under the microscope in median vertical section (fig. 6 *a*). There are a distal light zone, in which phagocytosis predominates and in which numerous macrophages of reticulum cell origin are seen filled with degenerated lymphocytes, and a proximal dark zone, in which medium-sized lymphocytes predominate, mitosis of these cells is prevalent and there are some macrophages. The secondary nodule is capped peripherally by a dense band of lymphocytes predominantly of the small variety.[11a] The photograph of a median vertical section of a whole anterior cervical node (fig. 5 *a*) and that of a median vertical section of a secondary nodule (fig. 6 *a*) illustrate these relations. Qualitatively there was little change from this characteristic histologic pattern in the nodules of the control group throughout the period, but in all of the poisoned groups the distinction between the zones of the nodules was lost and there were no sharp lines of demarcation between the nodules and the surrounding cortex. The nodules were looser and less lymphoid and appeared degenerated. The general trend of change is shown in the photographs of sample nodules from the TCA group (fig. 6 *b* to *e*).

In order to have histologic bases for contrasts of the conditions in the nodules, counts were made of the total number of cells and of the different types of cells per unit volume (90,000 cubic microns) of one nodule from one node of each rat used; and of the number of medium-sized lymphocytes in mitosis in one unit volume of each of ten nodules from one node of each rat used (fig. 4). These data were used with those of the weight of the nodes and the relative volume of the nodules to calculate the incidence of each of the different types of cells and of cells in mitosis per hundred grams of body weight. Since the calculated data convey a better idea of the trend of change, it was considered unnecessary to include all of the raw data in this paper. Data not included are on file in this laboratory and in the files of the Medical Division of the Chemical War Service.

Examination of the data in table 4 A shows that in the control group there was no significant change in the average number of cells per hundred grams of body weight in the secondary nodules throughout the four day period. However, in all of the poisoned groups there were initial significant reductions in the average numbers of cells. The greatest decrease occurred in the TCA group. In the nitrogen mustard groups there were no further reductions in the number of cells throughout the period, but in the SM group there was further significant decrease on the second day followed by a rise on the third and fourth days.

There was no significant change in the number of large lymphocytes in the control group until the fourth day, at which time the number increased (table 4 B). In the poisoned groups there was marked initial significant hypoplasia of large lymphocytes in the SM and TCA groups. Hypoplasia occurred in the ECA group on the second day and in the MCA group on the fourth day. In the SM group there was a trend toward increase during the remainder of the period, but in the TCA group there was no significant change after the second day.

The medium-sized lymphocytes did not change significantly in number in the controls throughout the period (table 4 C). In the poisoned groups there was marked significant hypoplasia of these cells in all groups. It was greatest in the TCA group. The hypoplasia persisted in the ECA and TCA groups throughout the period. In the SM and MCA groups there were significant increases in the

11a. Kindred, J. E.: Am. J. Anat. **62**:453, 1938.

numbers of medium-sized lymphocytes on the third day, and the increases continued through the fourth day.

The small lymphocytes were so variable in distribution that the only significant change occurred in the MCA group, where these cells were less numerous on

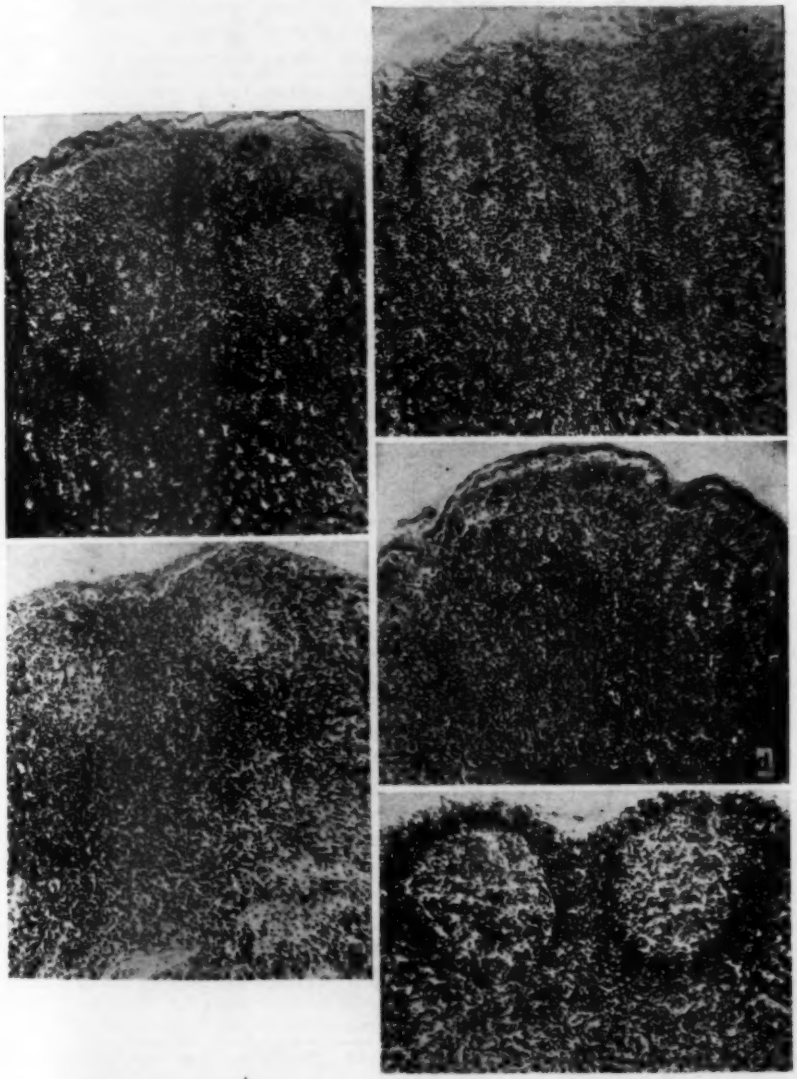

Fig. 6.—Photomicrographs of median vertical sections of secondary lymphoid nodules of anterior cervical lymph nodes, from sections shown in figure 5; × 58. (*a*) Control one day after injection of saline solution. (*b*, *c*, *d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

the second day than on the first, although the number was not significantly different from that in the control group (table 4 D).

Degenerated lymphocytes fluctuated in number in the controls (table 4 E). Practically all of them were within macrophages. Among the poisoned groups, initially the SM and MCA groups had numbers that were not different from the

TABLE 4.—*Secondary Lymphoid Nodules of the Anterior Cervical Lymph Nodes of the Rats Used for the Study of the Thymus and Distributed in the Same Way in the Different Groups*

The counts recorded are the average numbers of cells of different types ($\times 10^4$) and of medium-sized lymphocytes in mitosis ($\times 10^3$) in secondary nodules per hundred grams of body weight. The data were calculated from the data in figure 4 and from unlisted data on large and small lymphocytes, reticulum cells and macrophages. Each average is accompanied by its standard error.

Days	Saline Solution	SM	ECA	MCA	TCA
		A. Cells of All Types			
1	5,600 ± 780	3,470 ± 730*	2,550 ± 250*	2,650 ± 420	900 ± 292*
2	4,000 ± 560	1,200 ± 360†	1,400 ± 740	2,000 ± 260	1,200 ± 625
3	4,200 ± 455	3,450 ± 1,200	1,780 ± 630	2,120 ± 800	720 ± 200
4	5,100 ± 1,070	3,110 ± 580	.....	2,000 ± 360	1,380 ± 272
		B. Large Lymphocytes			
1	227 ± 58	30 ± 11*	143 ± 40	44 ± 8*	24 ± 16*
2	291 ± 81	65 ± 32	50 ± 29†	154 ± 46	37 ± 23
3	144 ± 25	90 ± 25	78 ± 30	150 ± 47	47 ± 20
4	300 ± 50†	120 ± 40	.....	90 ± 26	50 ± 9
		C. Medium-Sized Lymphocytes			
1	3,180 ± 485	421 ± 100*	618 ± 160*	456 ± 268*	94 ± 41*
2	2,060 ± 330	240 ± 113	207 ± 120	300 ± 48	420 ± 302
3	2,470 ± 515	1,800 ± 840†	500 ± 160	1,300 ± 500†	360 ± 88
4	3,440 ± 615	1,722 ± 475	.....	1,010 ± 180	862 ± 265
		D. Small Lymphocytes			
1	1,081 ± 380	1,860 ± 735	880 ± 221	1,200 ± 261	377 ± 146
2	1,166 ± 200	620 ± 148	812 ± 420	600 ± 181†	420 ± 192
3	650 ± 111	700 ± 163	810 ± 610	327 ± 99†	183 ± 90
4	466 ± 190	710 ± 310	.....	690 ± 170	260 ± 60
		E. Degenerated Lymphocytes			
1	734 ± 110	720 ± 170	2,690 ± 125*	660 ± 140	253 ± 76*
2	390 ± 49†	200 ± 61†	186 ± 100†	900 ± 122	170 ± 130
3	810 ± 150†	700 ± 210†	235 ± 37	261 ± 86†	90 ± 32
4	638 ± 268	400 ± 102	.....	100 ± 22	135 ± 31
		F. Medium-Sized Lymphocytes in Mitosis			
1	910 ± 102	30 ± 13*	112 ± 68*	22 ± 13*	2 ± 2*
2	740 ± 130	48 ± 16	36 ± 45	88 ± 27†	66 ± 605
3	610 ± 129	460 ± 190†	216 ± 625	360 ± 170	99 ± 43
4	1,070 ± 192	480 ± 220	.....	280 ± 85	270 ± 60†
		G. Reticulum Cells			
1	155 ± 38	235 ± 70	93 ± 43	140 ± 115	31 ± 9*
2	110 ± 61	54 ± 10†	100 ± 42	68 ± 18	80 ± 20
3	60 ± 16	54 ± 19	13 ± 10	27 ± 19	20 ± 12†
4	70 ± 19	48 ± 16	.....	50 ± 12	25 ± 7
		H. Macrophages			
1	180 ± 25	208 ± 39	175 ± 112	154 ± 49	116 ± 44
2	80 ± 24*	43 ± 13†	25 ± 8†	70 ± 40	24 ± 8
3	132 ± 23	120 ± 32	70 ± 26	70 ± 22	21 ± 5
4	166 ± 42	166 ± 25	.....	50 ± 10	50 ± 7†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

number in the control group, but in the ECA group there was an initial increase and in the TCA group a decrease. On the second day the numbers of all but the MCA group decreased. On the third day there occurred increase in the SM group, no change in the ECA and TCA groups and decrease in the MCA group.

There were no significant changes on the fourth day in the surviving rats. Calculations showed that the number of lymphocytes lost could not be accounted for by the degenerated cells; hence some of this loss must have been due to rapid emigration of cells or to the macrophages' rapid digestion of injured cells.

There was no significant change in the number of medium-sized lymphocytes in mitosis in the control group throughout the period (table 4 F). In all the poisoned groups, however, there was marked significant decrease of the numbers of lymphocytes in mitosis. The decrease was greatest in the TCA group. Significant recovery of mitotic activity began in the MCA group on the second day. On the third day there was significant increase in the SM group and in some rats of the ECA and TCA groups. It did not reach significant proportions in the TCA group until the fourth day. Because there was greater proportional decrease initially in the numbers of medium-sized lymphocytes in mitosis than in the numbers of medium-sized lymphocytes, it is concluded that mitotic activity was inhibited for at least two days in all but the MCA group.

The number of unmodified reticulum cells did not change in the control group throughout the period (table 4 G). In the poisoned groups, the number decreased initially only in the TCA group. It fluctuated in this group after the first day, but reticulum cells were always less numerous than in the controls. In the SM group there was a marked decrease on the second day, and in the ECA and MCA groups on the third day. Unlike those noted in the thymus, these scattered decreases did not occur because these cells became macrophages, for there were no initial increases in the numbers of macrophages such as were seen in the thymus (table 4 H). The macrophages tended to decrease in the poisoned groups, but the individual variations were so great that one can conclude only that there is a greater trend toward decrease than toward stability. This trend also occurred in the thymus. A contrast of the data concerning the macrophages of the lymph nodes and of the thymus indicates that there is a physiologic difference between the macrophages. In the lymphoid nodules the macrophages are always present and are continually selecting, ingesting and digesting injured lymphocytes, while in the thymus phagocytosis is apparently a secondary function and is brought about by the production of phagocytosis-stimulating substances around unmodified reticulum cells. This substance is apparently found around degenerating cells, a fibrin-like deposit which makes the surface of the injured cell sticky. These sticky cells when coming into contact with a reticulum cell may stimulate it to become a phagocyte. Another difference between the macrophages of the thymus and those of the lymphoid nodules was the accumulation of degenerated cells in the macrophages of the thymus and the little increase of the number normally seen in the macrophages of the lymphoid nodules. The absence of accumulations of injured lymphocytes in the macrophages of the lymphoid nodules is thought to be caused by their rapid ingestion and digestion. Such a view is based on the belief that these macrophages normally contain an active cytolytic enzyme. Many of the macrophages in both the thymus and the lymphoid nodules perished as a result of the burden imposed on them by the local destruction of cells. This seemed to be particularly true on the second day after injection. From examination of the relation of the macrophages and the degenerated cells it is believed that in both the thymus and the lymph node the disintegration of macrophages and the reduction in their number were not the result of direct toxic action of the agents but were due to exhaustion following excessive phagocytosis.

As a result of the destruction of the larger lymphocytes and the disintegration of some of the macrophages, the lymphoid nodules became very loose, and there were wide edematous spaces between the cells (fig. 6 *b*). However, there was no

complete necrosis, and after the wave of destruction had passed, the nodules again became more compact and regeneration began (figs. 6*d* and 8). Regeneration of the larger lymphocytes of the nodules could be from two sources: small lymphocytes which had been temporarily inhibited from growing by the action of the agents and reticulum cells which had been uninjured by the agents. There appears to be more evidence from the distributions of the small lymphocytes in the nodules on the third and fourth days for the first view.

In brief, it appears that the agents caused destruction of the larger lymphocytes in the secondary nodules of the lymph nodes, inhibition of mitosis in these cells and inhibition of growth of smaller into larger forms. The macrophages rapidly ingested and digested the injured cells, and many perished as a result of this activity The nodules became small, edematous and loose as a result of these changes. The toxic effects of the agents lasted for about two days and were followed by the beginning of regeneration of medium-sized lymphocytes and resumption of mitosis. The changes in the nodules of the lymph nodes paralleled the changes in the cortex of the thymus and the changes in the blood picture. While the lymphopenia of the blood was at its height, the destruction of lymphocytes and the inhibition of mitosis were greatest. These changes in the thymus and the lymphoid nodules are thought to be directly related to the lymphopenia of the blood. That there was not complete lymphopenia during this period of inhibition of lymphopoietic activity is thought to have been due to the fact that uninjured lymphocytes migrated from these organs. Migration of uninjured lymphocytes is believed to be the chief reason for the shrinkage of the thymus and the lymph nodes.

The medullas of the lymph nodes shrank in proportion to the shrinkage of the whole organs in the poisoned groups (fig. 5). These decreases probably occurred as lymphocytes migrated from the medullary cords. The plasma cells which form the bulk of the population of the medullary cords were apparently uninjured by the agents.

THE SPLEEN

In the normal rat the spleen plays a minor role in the hemopoietic system. The active lymphocytopoietic centers (malpighian nodules) produce relatively small numbers of lymphocytes compared with the thymus and the cervical lymph nodes. Granulocytes and erythrocytes and blood platelets are also produced in the spleen, but the numbers of these cells contributed to the circulating blood are small.[1] The spleen was studied in detail, however, by the same methods which were used for the study of the thymus and the lymph nodes, in order to ascertain whether the tissues had been affected in the same manner by the vesicants as had those of the thymus and the lymph nodes. Since the spleen plays such a minor role in hemopoiesis, it was decided not to include the detailed tables which were made from the quantitative study. It was felt, however, that a brief résumé should be given concerning the histologic conditions observed in the spleens of the poisoned rats. The most important trends of the changes observed in the spleen as a whole and in the splenic nodules are shown in figure 7.

The average weight of the spleen decreased significantly in the control group during the first two days after injection, but on the third and fourth days showed a trend toward increase (fig. 7). In all of the poisoned groups the weight of the spleen decreased significantly one day after injection and remained below the control level throughout the period. The relative volume of the lymphoid tissue was initially reduced in the poisoned groups but regenerated by the fourth day in all but the TCA group (fig. 7). The decrease of the weight of the spleen was

probably caused not by the decrease of the lymphoid tissue but by the contraction of the sinuses, which occurred in all of the poisoned groups and which was frequently accompanied by fibrosis. Red blood cells which normally occupy the sinuses and the intersinal spaces of the spleen were few in the poisoned rats, and the reticulum cells were contracted and formed dense masses. The changes in the size of the spleen are shown in the photographs of sections taken at the level of the hilus (fig. 8).

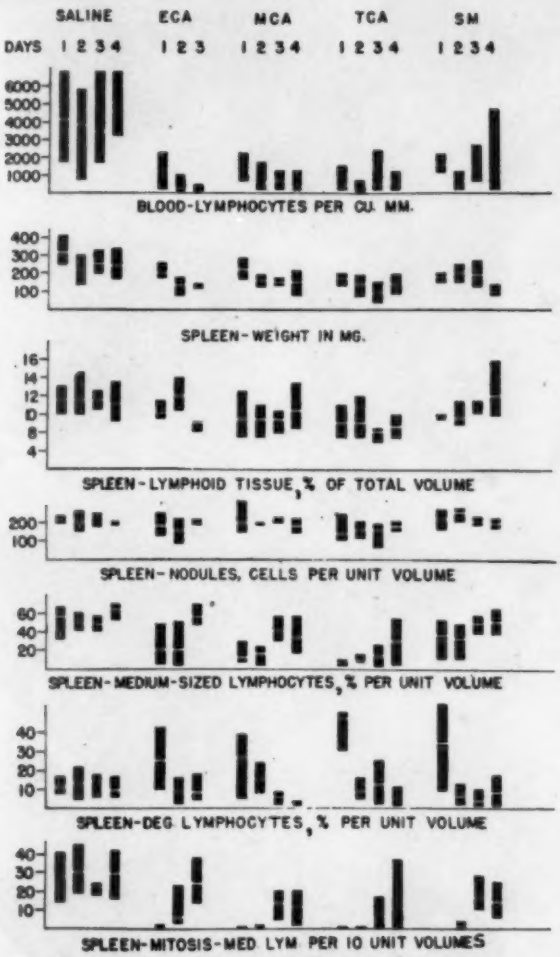

Fig. 7.—Block diagrams showing distributions of lymphocytes of the circulating blood, the weight of the spleen, the volume of the lymphoid tissue of the spleen and the cell counts of secondary lymphoid nodules of the spleen of rats one to four days after injection of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

As in the thymic cortex and in the cortex of the lymph node outside of the nodules the splenic lymphoid cords of the poisoned rats showed initial hypoplasia of lymphocytes, which resulted from local destruction of lymphocytes and loss due to migration. The degenerated lymphocytes were ingested and digested by macro-

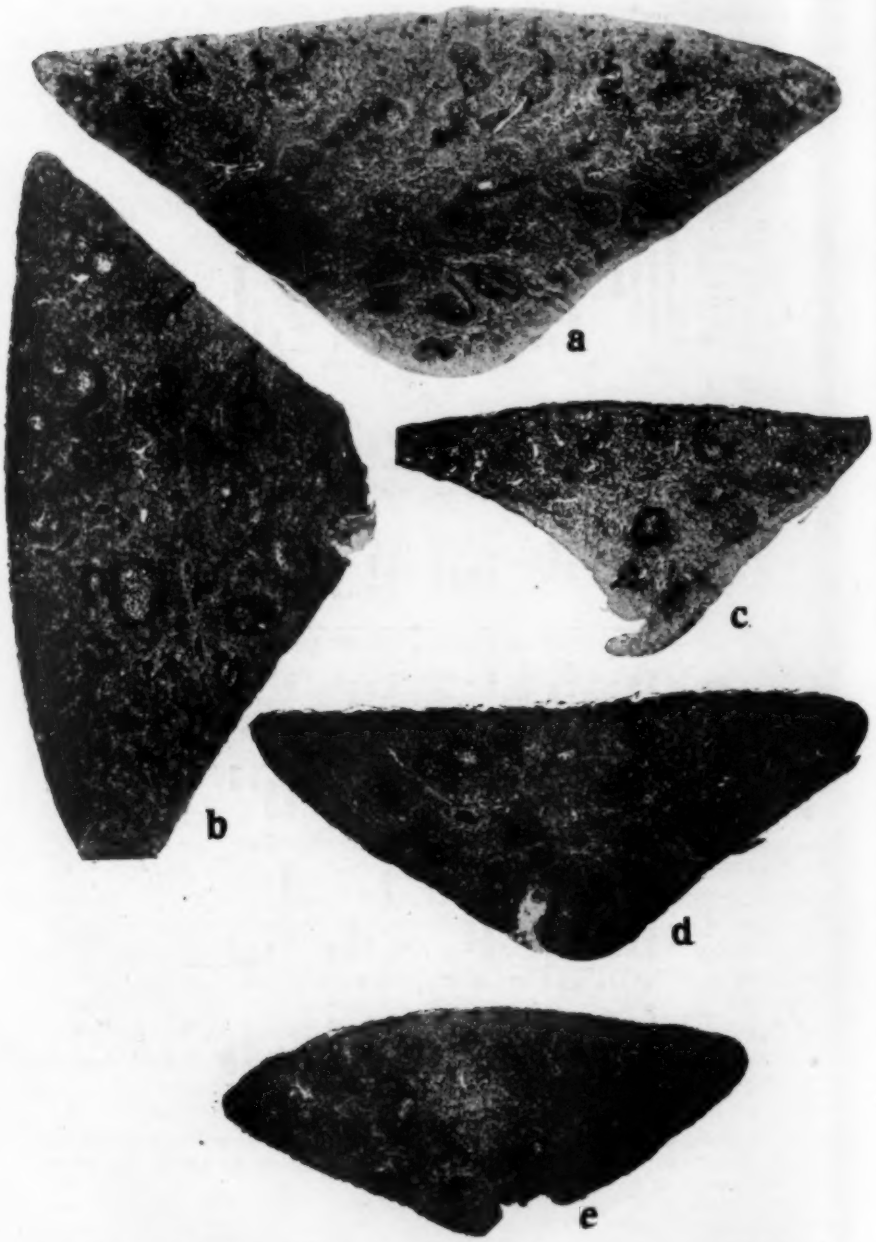

Fig. 8.—Photomicrographs of transverse sections of the spleen taken at the level of the hilus; hematoxylin and eosin; sections 7 microns thick; × 16. (*a*) Control one day after injection of saline solution. (*b, c, d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

phages of reticulum cell origin. Normally, in this part of the spleen there is little destruction, and few active macrophages are present. As a result of the loss of lymphocytes, the stroma became loose, and reticulum cells and blood capillaries were conspicuous (fig. 9 *b* to *e*). The cords became narrow and were often invaded by fibroblasts. The small degree of mitosis normally present was inhibited. Regeneration of large and medium-sized lymphocytes began on the

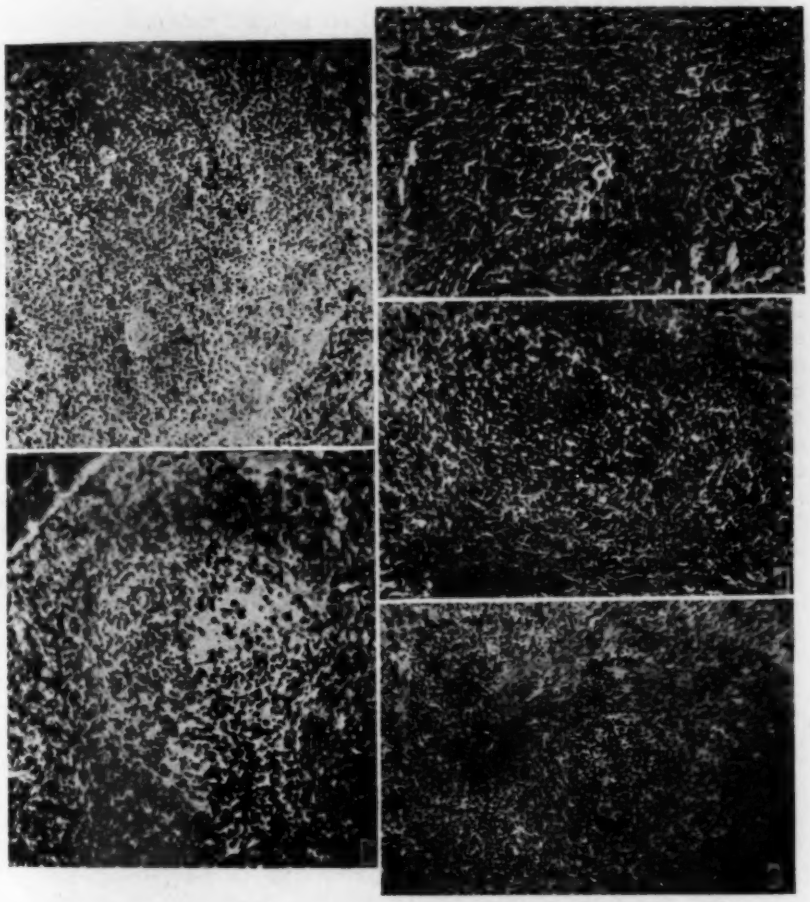

Fig. 9.—Photomicrographs of median vertical sections of secondary lymphoid nodules and adjacent lymphoid cords from sections shown in figure 8; × 54. (*a*) Control one day after injection of saline solution. (*b, c, d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

third day, but the number of small lymphocytes continued to decrease throughout the period. Mitotic activity was resumed in all poisoned groups by the third day but remained significantly lower than in the control group throughout the period. The TCA group showed the slowest regeneration. The reticulum cells were

reduced in number, possibly by the edema which separated them as the lymphocytes were destroyed or migrated, but probably the decrease was caused by their being transformed into macrophages. Throughout the later part of the period there was no consistency in the distribution of reticulum cells and macrophages. It is possible that this inconsistency was due to the degeneration of the macrophages following their novel phagocytic activity. On the whole there is marked similarity between the changes occurring in the splenic cords and those in the thymic cortex and the cortex of the lymph node.

The splenic nodules (malpighian nodules) are normally sites of a minor degree of destruction and proliferation of lymphocytes. They contribute few lymphocytes to the general circulation.[1] There are no light and dark zones in these nodules such as are present in the nodules of the lymph nodes, and the nodules are surrounded by a dense zone of unmodified reticulum cells (fig. 9 *a*). These histologic conditions were maintained in the control rats. In the poisoned rats there was hypoplasia of lymphocytes in the nodules and increase of the number of degenerated lymphocytes in the macrophages (fig. 7). The nodules became loose, and the reticulum-macrophage stroma became prominent. Photographs of samples from the TCA group illustrate these initial changes (fig. 9 *b*). The peripheral zone of reticulum cells loosened and was infiltrated by red blood cells from the neighboring sinuses. Mitosis of the medium-sized lymphocytes was inhibited (fig. 7). Regeneration of the nodules, characterized by increase in the number of medium-sized lymphocytes and resumption of mitosis, began on the third day (fig. 7). At the same time degenerated lymphocytes and macrophages decreased in numbers, and the surrounding reticular stroma became more compact (fig. 9 *c* to *e*). The sequence of destruction and regeneration paralleled, both in general character and in time, that which occurred in the nodules of the lymph nodes except in one respect. This particular difference was in the initial increase of the number of degenerated lymphocytes contained within the macrophages of the splenic nodules, whereas it will be recalled that degenerated cells decreased or did not change significantly in number in the macrophages of the lymphoid nodules of the lymph nodes. Such a difference suggests that the cytolytic enzymes of the macrophages of the splenic nodules are not as active as those of the macrophages of the lymph nodes.

The granulocytopoietic and erythrocytopoietic areas did not change in volume in the controls throughout the period. But in the poisoned rats there were reductions in the numbers and in the mitotic activity of the myelocytes and the erythroblasts for two days after the injection. The reduction in the numbers of cells appeared to be due to loss by migration since there was no evidence of local destruction of cells such as occurred in the lymphoid parts of the spleen. The megakaryocytes in these areas were unaffected by the agents.

The venous sinuses were collapsed or contracted in the poisoned rats and were mostly devoid of red blood corpuscles, which characterize these regions in the controls. In most of the rats the spleen had large areas of fibrosis, which originated from the hilus. In some rats the whole spleen was infiltrated with fibroblasts. Despite these changes there was no evidence of localized necrosis. There was no evidence of increased destruction of red blood corpuscles.

In brief, the lymphoid tissue of the spleen experienced the same kind of degeneration which characterized the lymphoid tissue of the cortices of the thymus and the lymph nodes; in addition its function as an accessory myeloid and erythroid center was temporarily depressed. Likewise, its function as a storage reservoir for red blood corpuscles was reduced.

COMMENT ON THE LYMPHOID ORGANS

In table 5 are presented in brief form contrasts between the numbers of lymphocytes of the blood and the amounts of lymphoid tissue observed in the thymic cortex, in the secondary nodules of the cervical lymph nodes and in the spleen in the poisoned rats on the first and fourth days after injection. These data are given in terms of percentage of the average value of each item as obtained for the control rats at the end of the first day after injection. The purpose of the table is to contrast briefly the degrees of reduction of the lymphoid tissue of the several organs caused by the vesicants.

It will be seen in table 5 that sulfur mustard produced the least initial effect on the number of lymphocytes of the blood and on the

TABLE 5.—*Percentages of First Day Control Values to Which Lymphocytes of Blood and Lymphoid Tissues of Thymic Cortex, Secondary Nodules of Cervical Lymph Nodes and Lymphoid Tissue of Spleen Had Been Reduced on First and Fourth Days, Respectively, After Injection of Vesicants* *

Cells or Organ	Day	Saline Solution	SM	ECA†	MCA	TCA
Lymphocytes.....	1	100 ± 7.2	42 ± 5.1	27 ± 10.2	27 ± 3.9	11 ± 2.0
	4	111 ± 16.0	21 ± 14.0	8 ± 1.5	11 ± 1.0	13 ± 2.8
Thymus.....	1	100 ± 3.5	50 ± 10.5	60 ± 3.0	55 ± 6.1	50 ± 10.5
	4	56 ± 6.5	16 ± 1.6	19 ± 1.9	25 ± 2.5	16 ± 1.6
Lymph nodes.....	1	100 ± 10.0	63 ± 9.2	50 ± 14.0	46 ± 8.8	21 ± 6.2
	4	87 ± 13.0	52 ± 12.5	25 ± 8.8	37 ± 6.2	23 ± 3.7
Spleen.....	1	100 ± 15.0	80 ± 14.5	62 ± 6.0	64 ± 11.0	44 ± 4.9
	4	80 ± 14.5	54 ± 25.0	35 ± 3.1	62 ± 7.0	39 ± 18.0

* Each value is followed by its standard error.
† The values in the ECA group are given for the third day, since there were no survivors beyond this day.

amounts of lymphoid tissue of all organs except the thymus; tris (2-chloroethyl) amine had the greatest effect, while ethyl-bis (2-chloroethyl) amine and methyl-bis (2-chloroethyl) amine produced almost identical effects intermediate between those of sulfur mustard and tris (2-chloroethyl) amine. The lymphoid tissue of the thymic cortex was affected initially less than that of the spleen in all except the ECA group. In all but the SM group, however, the secondary lymphoid nodules of the lymph nodes were reduced by a greater degree than either the lymphoid tissue of the thymic cortex or that of the spleen. By the fourth day the lymphoid tissue of the thymic cortex had been reduced by a greater degree in all groups than that of either the spleen or the lymph nodes. The greatest relative reduction occurred in the ECA group. The only significant further reduction of the lymphoid tissue of the lymph nodes and the spleen occurred in the spleen in the ECA group.

In relating these changes to the changes in the number of lymphocytes of the circulating blood, it appears that the chief factor responsible for the drastic reduction of the number of lymphocytes which occurred was the decrease in the amount of lymphoid tissue of the thymic cortex and the depression of its lymphocytopoietic activity. The acute changes in the thymus may have been shared by the lymph nodes and the spleen, but the lymphocyte production of these organs is normally only a fraction of that of the thymus. A rough calculation based on the data concerning the incidence of cells in mitosis in the thymic cortex, the secondary nodules of the cervical lymph nodes and the spleen shows why this occurs. From these data it has been estimated that if it takes one hour for the completion of a mitotic cycle, the thymic cortex of the control rat could produce per hour about 10 per cent of the number of lymphocytes of the blood per hundred grams of body weight, whereas the cervical lymph nodes could produce about 3.0 per cent and the spleen about 0.4 per cent. At the end of the first day after the injection of the vesicants the number of lymphocytes produced by the thymic cortex, it has been estimated, would have dropped to such a degree that only about 0.4 per cent of the normal complement of lymphocytes would be produced per hour in the SM group, 0.7 per cent in the ECA group, 2.2 per cent in the MCA group and 0.2 per cent in the TCA group. In the secondary nodules of the cervical lymph nodes the estimated production dropped to about 0.09 per cent in the SM group, to 0.35 per cent in the ECA group, to 0.06 per cent in the MCA group and to 0.006 per cent in the TCA group. And in the secondary nodules of the spleen the drop was to 0 in the SM group, to 0.005 per cent in the ECA and MCA groups and to 0.002 per cent in the TCA group. Thus it is evident that the lymphopenia of the poisoned rats is largely due to the decrease of the number of lymphocytes produced by the thymic cortex and only to a lesser degree to the decrease of the numbers produced by the lymphoid tissue of the cervical lymph nodes and the spleen, even though the lymphoid tissue of the thymus did not decrease relatively as much as did that of the cervical lymph nodes and that of the spleen. Furthermore, the decrease of the numbers of lymphocytes of the circulating blood of the poisoned rats was of the same degree as that observed in rats from which all of the lymphoid tissue (93 per cent) except that of the alimentary tract [12] had been removed. It has been shown, moreover, that the spleen plays a minor role in relation to the lymphocytes of the blood of the rat, since there is no change in the count of the white cells of the peripheral blood of rats [13] after splenectomy.

The injection of the adrenocorticotropic hormone of the pituitary gland, which causes acute hypoplasia of the thymus, the spleen and the

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lymph nodes, is followed by an acute numerical decrease of the lymphocytes of the circulating blood in the mouse [14] and in the rat and the dog.[15] From the original investigations of Heineke [16] and many later investigations [17] it has been shown that in small animals exposed to roentgen rays acute degenerative changes develop in the lymphocytes of the thymus, the lymph nodes and the spleen, accompanied by lymphopenia of the blood. Thus the belief that the lymphopenia of the rats poisoned by injected vesicants is caused by degenerative changes of the lymphoid organs, particularly of the thymus, receives support from others' investigations of the relation between acute degeneration of the lymphoid organs and lymphopenia of the blood.

The same acute degenerative effects are produced in the thymus and the lymph nodes by agents which cause the "alarm reaction" in rats,[18] and they result in transient lymphopenia of the blood.[19] The acute degenerative changes caused by compounds which cause the "alarm reaction" are prevented by adrenalectomy,[20] as are those caused by the adrenocorticotropic hormone.[21] Likewise, the lymphopenia which accompanies these changes does not occur in adrenalectomized animals.[22] According to these investigators, the cortex of the adrenal gland is the mediator of the substance which produces the degenerative effect. In animals treated with compounds causing the "alarm reaction" the adrenal cortex hypertrophies and the lipoid content decreases.[20] In animals given injections of adrenocorticotropic hormone there is depletion of the sudanophilic material and of the cholesterol of the adrenal cortex.[23] In dogs irradiated with roentgen rays there is congestion of the cortex,[24] and in rabbits also.[25] No change was found in the adrenal glands of mice which

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had been treated with roentgen rays or neutrons,[26] but after irradiation of rats the cells of the adrenal cortex hypertrophied and lost fat.[27] In rabbits poisoned by injected sulfur mustard there was acute congestion of the adrenal glands,[4b] and in rabbits in which the skin had been contaminated with sulfur mustard there was decrease of cortical lipoid materials.[28] In rats receiving injections of methyl-bis (2-chloroethyl) amine there were 60 per cent increase of the weight of the adrenal glands, hypertrophy of the cortex and depletion of the lipoid.[29] In the rats used in the present experiments the adrenal glands were not hypertrophied, but there was increase in the size of the cells of the spongy zone of the zona fasciculata and decrease in the number of fat vacuoles of the adrenal cortex. Ludewig and Chanutin[30] observed increase of weight with decrease of cholesterol in the adrenal glands of a larger group of rats from this same colony which had received injections of vesicants. Thus it appears that the lymphoid changes accompanying injections of compounds causing the "alarm reaction," roentgen irradiation and other treatments and interpreted as having been caused by changes occurring in the metabolism of the cells of the adrenal cortex are paralleled by similar morphologic and physiologic changes in the adrenal glands of rats poisoned with vesicants. As pointed out, the degenerative changes following the administration of compounds causing the "alarm reaction" and that of adrenocorticotropic hormone are prevented by adrenalectomy. However, the degenerative changes following roentgen irradiation of rats are not prevented by adrenalectomy if the thymus is not shielded,[27] and adrenalectomized rats poisoned by injected methyl-bis (2-chloroethyl) amine show the same degenerative changes as do the nonadrenalectomized rats, although the effect may not be quite as great.[31] This decrease of effect was also noted by Leblond and Segal[20] in adrenalectomized rats which had been exposed to roentgen rays.

In the present investigation, adrenalectomized rats received injections of a variety of nonlethal doses of tris (2-chloroethyl) amine. The lymphoid organs showed degenerative changes of the same type and degree as those observed in the lymphoid organs of nonadrenalectomized rats, and the damage was proportional to the amount injected. Hence it is concluded that the vesicants produce some of their intoxicating effects on the lymphoid organs directly, although they in part may be

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31. Karnofsky, D. A.; Graef, I., and Smith, H. W.: Federation Proc. **5**:224, 1946.

mediated by the adrenal cortex. This conclusion is in agreement with that of Karnofsky and associates.[31]

At this point it seems pertinent to inquire into the mechanism by which the mustards act on the lymphoid cells. In a series of in vitro observations of lymphocytes of the thymus which were suspended in plasma to which tris (2-chloroethyl) amine had been added in a proportion equivalent to that obtaining in the solution injected, it was found that the nuclei of the cells were injured within two hours after the beginning of the exposure. The nucleus first became opaque, and then the chromatin broke up into small vesicles which flowed together. Subsequently the nuclear membrane seemed to dissolve, and the chromatin globules were scattered through the cytoplasm. In fixed materials this same sequence of events was observed, and in sections treated with Feulgen's reagent the chromatin particles gave a positive reaction for desoxyribonucleic acid, in which the thymus is particularly rich.[32] It is thought that during the active life of the cell the desoxyribonucleic acid is being continually built up and broken down,[32] a metabolic condition which has been detected by the use of ultraviolet rays [32] and radioactive phosphorus.[33] The desoxyribonucleic acid is particularly unstable in cells which are actively dividing,[32] such as the medium-sized lymphocytes of the thymus and of the centers of the nodules of the lymph nodes and the spleen. The vesicants stop this activity. The question may be asked whether the agents damage the protein, the desoxyribonucleic acid or the enzyme system responsible for the maintenance of nuclear and cytoplasmic function. If the Feulgen reaction is taken to be a positive indication of the presence of desoxyribonucleic acid, the vesicants do not destroy this substance, as it still can be seen in the nuclei of degenerated cells even after they have been ingested by macrophages. Since the cells are no longer actively dividing, it is possible that the vesicants may depolymerize the desoxyribonucleic acid and deactivate it in much the same way as sulfur mustard has been reported to deactivate certain viruses.[34] Radium causes similar nuclear degeneration of cells in vitro [35]; and in vitro cells of the thymus of the rabbit undergo degeneration when exposed to roentgen rays, and the degeneration is proportional to the dose administered.[36] As yet, however, there has been no demonstration of all of the chemical changes which could account for

32. Greenstein, J. P.: Nucleoproteins, in Edsall, J. T., and Anson, M. L.: Advances in Protein Chemistry, New York, Academic Press, 1944, vol. 1, p. 209.

33. Andreasen, E., and Ottensen, J.: Acta path. et microbiol. Scandinav., 1944, supp. 54, p. 25.

34. Tenbroeck, C., and Herriott, R. M.: Proc. Soc. Exper. Biol. & Med. **62**:271, 1946.

35. Canti, R. G.: Acta radiol. **10**:320, 1929.

36. Schrek, R.: Radiology **46**:395, 1946.

the morphologic changes which have been observed in the nuclear chromatin of cells injured by physical and chemical agents. For discussions of the problems relating to these changes the reader is referred to Gilman and Phillips [5] and Greenstein.[32]

As has been stated, there is lymphopenia of the circulating blood at the same time that degenerative changes are going on in the lymphoid organs of the poisoned rats. The question arises as to the fate of the lymphocytes of the circulating blood. With the inhibition of mitosis, lymphocytes are not entering the blood stream in the numbers which are normally expected, and there is no evidence from the blood smears that they are degenerating in the blood stream. The fact that the uninjured lymphocytes are drained from the lymphoid organs is believed to account for the continued shrinkage of the lymphoid tissue of the several organs studied, and this drainage can prevent complete lymphopenia, but it does not account for the original loss of lymphocytes. Bunting and Huston [37] have suggested in regard to normal rabbits that the lymphocytes are lost from the body through the intestinal epithelium. Jordan [38] has suggested that the lymphocytes which are in excess of needs of the circulating blood are filtered off into the bone marrow, where they act as ancestors of the erythroblasts. Recently Farr [39] has shown that lymphocytes marked with acroflavine when introduced into the circulation of rabbits remain in the circulation for sixty to seventy-two hours and then disappear. Marked lymphocytes were found in the marrow and to a lesser extent in the lymphoid tissues, but no statement was made about their relation to the intestinal mucosa. In the rats which received injections of tris (2-chloroethyl) amine there were no accumulations of lymphocytes in such tissues as the lungs, the stomach, the trachea, the liver, the nervous system or the bone marrow, but large numbers of degenerated and uninjured lymphocytes were found in the lamina propria and in the lumen of the small intestine in regions where the epithelium of the villi had been sloughed off or where the epithelial cells had been injured. In the normal rat, it has been calculated, there could be $125{,}000{,}000 \pm 32{,}000{,}000$ small lymphocytes in the intestinal epithelium per hundred grams of body weight.[1] This number amounts to about three times the number of lymphocytes estimated to be present in the circulating blood at any one time. It was thought that in the normal rat these lymphocytes could not pass through the epithelium because of the intercellular cement present between the termini of the epithelial cells.[40] Recently Andrew and Andrew [41] have suggested that the lymphocytes

37. Bunting, C. H., and Huston, J.: J. Exper. Med. **33**:593, 1921.
38. Jordan, H. E.: Anat. Rec. **73**:227, 1939.
39. Farr, R. S.: Anat. Rec. **94**:460, 1946.
40. Chambers, R., and Renyi, G. S.: Am. J. Anat. **35**:385, 1925.
41. Andrew, W., and Andrew, N. V.: Anat. Rec. **93**:251, 1945.

escape by this route in the normal mouse; in the rat such a fate has been suggested.[42] Since in the normal rat great numbers of lymphocytes do occur in the intestinal mucosa, and since this mucosa is injured by the vesicants, it is believed that the initial lymphopenia results because lymphocytes are lost through this region. Eosinophilic granulocytes also seemed to be leaving the body through this region. Hence it is suggested that the decrease in their incidence noted in the poisoned rats may be partially explained by their leaving through this route.

In animals treated with compounds causing the "alarm reaction" enteritis occurs,[18] and since in animals subjected to roentgen irradiation there is distinct injury of the intestinal mucosa [43] and the other changes described in the vesicant-injured animals are characteristic of animals irradiated with roentgen rays, including lymphopenia, it is suggested from the nature of the syndrome that the lymphocytes are lost from the body through the intestinal mucosa. Other investigators have found the intestinal mucosa to be damaged after intoxications due to sulfur mustard,[44] ethyl-bis (2-chloroethyl) amine,[44c] methyl-bis (2-chloroethyl) amine [45] and tris (2-chloroethyl) amine,[46] respectively. In man the lymphopenia following injection of methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine is accompanied by nausea.[47]

THE FEMORAL MARROW

The marrow of the femur of the normal rat is dense; there is little fat, and few sinusoids are discernible. The marrow of the control group was like this (fig. 11 *a*). In the control group there was no change in the average number of cells per unit volume (50,000 cubic microns) throughout the four day period (fig. 10 and table 6 *A*). In the poisoned groups the marrows were slightly hypoplastic and more hyperemic than the marrow of the control group at the end of the first day (fig. 11 *b*). The greatest hypoplastic changes occurred in the TCA and SM groups (fig. 10 and table 6 *A*). Changes of this type were less marked in the ECA and MCA groups, although there was individual variation. The numbers of cells were significantly reduced in the SM and TCA groups. On the second day there was marked significant cellular hypoplasia with concomitant hyperemia in all the poisoned groups (fig. 11 *c*), and the average numbers of cells per unit volume were significantly less than the control average. In sections of the marrow

42. Andrew, W.: Anat. Rec. **94**:447, 1946.

43. Warren, S. L., and Whipple, G. H.: (*a*) J. Exper. Med. **35**:187 and (*b*) 202, 1922; (*c*) **38**:741, 1923. Shouse and others.[24] Lawrence and Tennant.[26]

44. (*a*) Courtice, F. C., and Cameron, G. R.: Unpublished data, 1942. (*b*) Cameron, G. R., and Short, R. H. D.: Unpublished data, 1942. (*c*) Graef, I.; Karnofsky, D. A.; Jager, J. R., and Smith, H. W.: Federation Proc. **5**:221, 1946; unpublished data, 1945.

45. (*a*) Crawford, B., and Smith, H. W.: Unpublished data, 1944. (*b*) Graef and others.[44e]

46. (*a*) Short, R. H. D.: Unpublished data, 1943. (*b*) Hanck, C. R.: Unpublished data, 1944.

47. Goodman and others.[6b] Jacobson and others.[6c]

the cells were scattered between the enlarged sinusoids, and there were large areas in which only cell remnants, fibrin and stroma could be seen. These changes produced a reduction in the amount of active marrow (fig. 10). Red blood corpuscles occupied many of these spaces, and it appeared as though these spaces

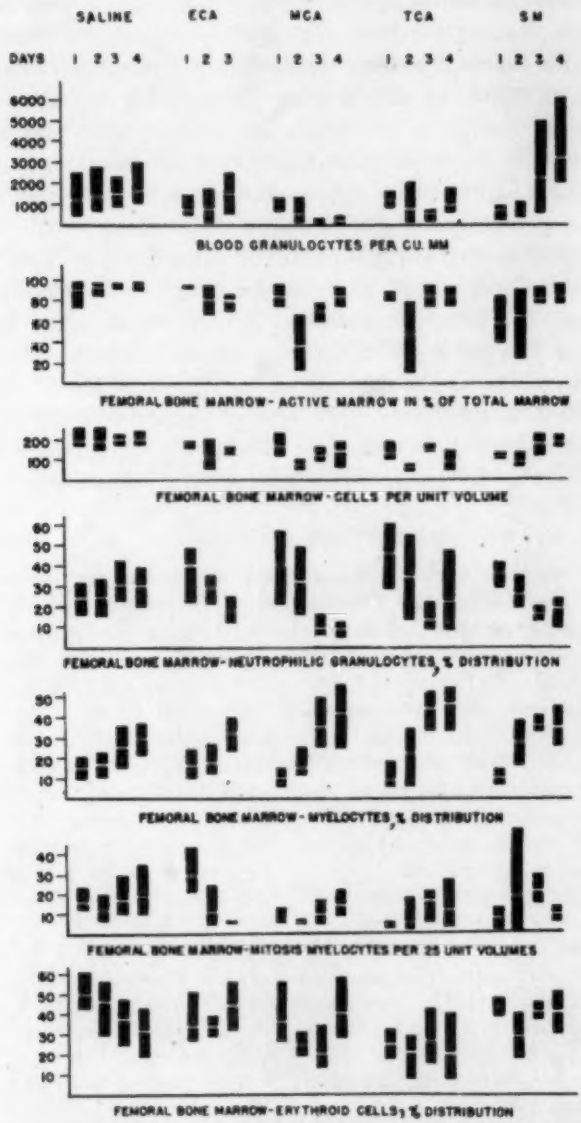

Fig. 10.—Block diagrams showing distributions of the neutrophilic granulocytes of the circulating blood, the percentage volume of active marrow and distributions of cells of the femoral marrow of rats one to four days after injection of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

were continuous with the sinusoids. The cells for the most part were uninjured, but there were some cells with pyknotic nuclei. In some marrows there were masses of fibrin, a sure sign of cellular injury. The reticulum cells and the endothelial cells were prominent, and the characteristic grouping of erythroblasts into scattered islands was absent. The osteoblasts on the endosteum were unchanged. The marrow when removed to make smears had a watery consistency. Conditions more or less like this persisted in the ECA, MCA and TCA groups throughout the period, but there were significant increases in the cell populations toward the end of the period, although they still remained below the normal level. In the SM group the normal denser character of the marrow was present in some rats on the third and fourth days. The typical conditions on the first through the fourth day are shown in the photographs of sample sections from the marrow of the TCA group (fig. 11 *b* to *e*).

A quantitative study was made of the cell populations with particular reference to myelocytes, neutrophils, nucleated erythroid cells and cells in mitosis. The percentage distributions of the cells were calculated from smears (fig. 10), and the numbers of cells per unit volume and the number of cells in mitosis, from sections (fig. 10). The data on the cell distributions per unit volume given in table 6 were calculated from these counts.

The myelocytes increased significantly in number in the control group on the third day and remained significantly high on the fourth day (fig. 10 and table 6 *B*). In the SM and MCA groups they were less in number than in the control on the first day. On the second day they had decreased in the ECA and TCA groups, remained low in the MCA group and increased in number in the SM group. In all groups there were significant increases in number on the third day, so that the average numbers were equal to that of the control group and remained so on the fourth day.

Normally there are not many myelocytes in mitosis. In the control group there was no change in the number in mitosis throughout the period (fig. 10 and table 6 *C*). In the SM, MCA and TCA groups there were significant decreases in the numbers of these cells in mitosis, and it is thought that mitosis was inhibited. The inhibition of mitosis continued in the MCA group until the fourth day, in the TCA group until the third day and in the SM group until the second day. In the ECA group, in which there had been no change during the first two days, there was a significant decrease on the third day.

The neutrophilic granulocytes increased in number on the third and fourth days (fig. 10 and table 6 *D*) in the control group. In the poisoned groups the numbers were not significantly different from the number in the control group on the first day, but on the second day the numbers in the SM, MCA and TCA groups had been reduced significantly. On the third day the number in the SM group rose significantly, the numbers in the ECA and MCA groups decreased and the number in the TCA group did not change. The levels reached on the third day were characteristic of the fourth day. Thus, in contrast with the control, the poisoned groups suffered a significant decrease in mature neutrophilic granulocytes.

The nucleated erythroid cells did not change significantly in number until the fourth day in the control group, at which time they were significantly fewer than on the first day (fig. 10 and table 6 *E*). In all of the poisoned groups there were significant decreases on the first day and the decreases became more marked on the second day. On the third day the numbers in the SM and ECA groups approached control level, and the numbers in the MCA and TCA groups showed significant increases from second day values. These changes are believed to be

an indication of regeneration of these cells. These trends continued in the SM and MCA groups but slowed up in the TCA group.

There was no change in the number of erythroid cells in mitosis in the control or in the ECA group throughout the period (table 6 *F*). In each of the other groups the number of these cells in mitosis was significantly lower than the control average on the first day. The number reached control level in the SM

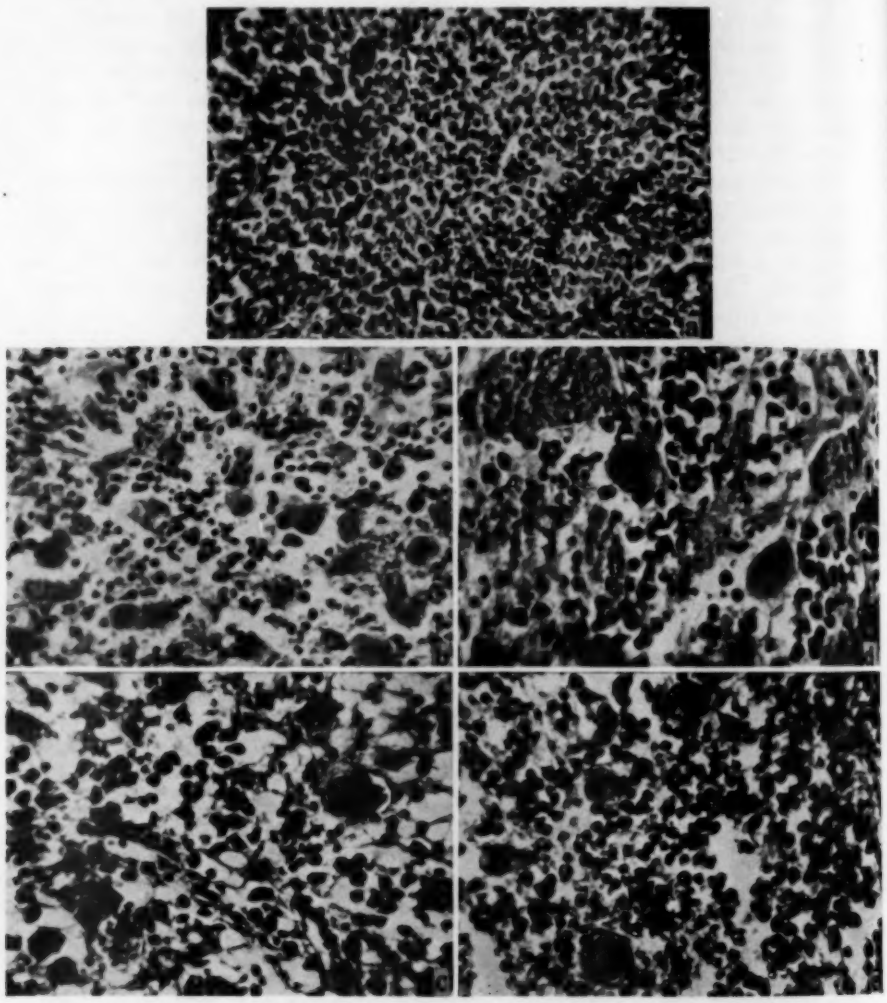

Fig. 11.—Photomicrographs of sections of femoral marrow; hematoxylin and eosin; section 5 microns thick; × 280. (*a*) Control one day after injection of saline solution. (*b*, *c*, *d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

group on the second day, in the TCA group on the third day and in the MCA group on the fourth day. Since the proportionate decrease in the number of cells in mitosis was greater than the decrease in cells, it is believed that

mitosis was inhibited during the first day in all of the poisoned groups except the ECA group.

The initial hypoplasia of the marrow in the poisoned groups appears to be due to decrease in the mitotic activity of the myelocytes and erythroid cells and to continued migration of matured elements. Although there was some destruction of granulocytes, calculated losses of cells could not be accounted for by the

TABLE 6.—*The Femoral Marrow from the Rats Which Were Used for the Study of the Lymphoid Organs*

The average numbers of different types of cells per unit volume (50,000 cubic microns) and the average numbers of myelocytes and erythroid cells in mitosis per 25 unit volumes are recorded as found one, two, three and four days, respectively, after injection. The distributions were calculated from the data in figure 10. Each average is followed by its standard error.

Days	Saline Solution	SM	ECA	MCA	TCA
	A. All Types of Cells				
1	190 ± 17	115 ± 7*	155 ± 9	162 ± 18	125 ± 13*
2	190 ± 15	85 ± 13†	105 ± 33	75 ± 9†	60 ± 8†
3	200 ± 5	190 ± 24†	140 ± 9	120 ± 11†	160 ± 10†
4	200 ± 8	170 ± 16	.....	140 ± 13	90 ± 11†
	B. Myelocytes				
1	28 ± 5	14 ± 1*	27 ± 9	15 ± 2*	21 ± 7
2	32 ± 4	28 ± 6	16 ± 4	10 ± 1†	11 ± 4
3	50 ± 3†	65 ± 7†	39 ± 4†	50 ± 9†	61 ± 13†
4	58 ± 3	56 ± 7	.....	57 ± 5	53 ± 5
	C. Myelocytes in Mitosis				
1	16 ± 2	6 ± 1.7*	16 ± 4	7 ± 1.1*	5 ± 2*
2	11 ± 2	15 ± 9	10 ± 5	5 ± 0.3	7 ± 3
3	16 ± 2.6	25 ± 2.5	5 ± 0.5	8 ± 2.4	16 ± 1.4†
4	19 ± 3	11 ± 1.5†	.....	13 ± 1.7	12 ± 3
	D. Neutrophilic Granulocytes				
1	45 ± 4	42 ± 3	60 ± 8	66 ± 14	44 ± 8
2	47 ± 5	21 ± 2.5†	34 ± 12	26 ± 8†	21 ± 5†
3	62 ± 3†	36 ± 3†	23 ± 5	11 ± 2.3	23 ± 8
4	60 ± 4	35 ± 5	.....	11 ± 1.4	27 ± 8
	E. Nucleated Erythroid Cells				
1	100 ± 10	50 ± 3.5*	57 ± 9*	60 ± 7*	33 ± 6*
2	87 ± 8	25 ± 3†	40 ± 13	17 ± 1.5†	11 ± 3†
3	80 ± 7	66 ± 14†	74 ± 13†	31 ± 2.5†	56 ± 13†
4	58 ± 6	67 ± 7	.....	53 ± 8†	22 ± 6†
	F. Nucleated Erythroid Cells in Mitosis				
1	5 ± 1.4	1 ± 0.4*	3 ± 1.2	1 ± 0.3*	1 ± 0.5*
2	6 ± 1.8	5 ± 2.7†	2 ± 2.0	0.4 ± 0.2	0.2 ± 0.2
3	5 ± 1.0	5 ± 1.5	4 ± 1.4	0.2 ± 0.2	8 ± 2.3†
4	4 ± 0.7	6 ± 2.0	.....	4.0 ± 0.4†	2 ± 0.4

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

destructive action of the agents. The agents seemed to retard growth and to inhibit mitosis and, as a consequence, the mature cells were not replaced as they migrated from the marrow. The second day seemed to be the peak of the hypoplastic conditions, and recovery was more rapid in the SM group than in the others.

The number of megakaryocytes did not change significantly in any of the groups throughout the period. Hemocytoblasts, mast cells and reticulum cells did not change in number, although there were relative increases in the number of reticulum cells because of the decreases in the populations of the other cells. Eosinophilic granulocytes tended to decrease in number in all of the poisoned groups on the last two days of the period. Plasma cells were always present

and did not seem to be damaged by the agents. The incidence of lymphocytes was small, but they never were numerous even in the control group.

On the whole the marrow did not undergo great destructive changes such as occurred in the lymphoid organs as a result of the intoxication following injection of vesicants. In both lymphoid organs and marrow there was cellular hypoplasia, but in the former a large part of the hypoplasia was due to injury of cells, whereas in the latter most of the hypoplasia was apparently caused by migration of matured elements without replacement. The erythroid elements of the marrow seemed to be depleted to a greater degree than were the myeloid elements.

It has been stated by Karnofsky and associates [29] that the cells of the marrow swell in rats poisoned with methyl-bis (2-chloroethyl) amine. This statement is hard to prove quantitatively since fixation changes may have some effect on the physical characteristics of injured cells. However, in order to test this view, camera lucida drawings were made under oil immersion magnification (1,700 diameters) of the largest of the several types of cells chosen at random from a number of fields in smear preparations. These drawings were then measured and the average diameters recorded. The average diameter of the largest hemoblasts in the control group of the first day was 13.8 microns. The average diameter of the largest hemoblasts in each group except the SM group exceeded that of the control. The average diameter of the largest myelocytes in the control group of the first day was 14.5 microns. This diameter was exceeded by the largest myelocytes in each of the poisoned groups on the first and later days. The diameters of the viable neutrophilic and eosinophilic granulocytes and that of the erythroblasts did not exceed the diameters of these cells in the control. Hence it is concluded in agreement with Karnofsky and associates [29] that swelling of the hemoblasts and myelocytes is characteristic of the marrow of rats injured by injected vesicants. This swelling may be the prelude to death, for at the end of the first day there were significantly fewer myelocytes in the SM and MCA groups than in the control, and on the second day, in the ECA and TCA groups. Furthermore, in the swollen cells the nuclei did not seem to be as sharply defined as those of the cells of normal size.

In the ECA group practically no degenerated cells were observed. But in the other groups, particularly in the MCA group, there was marked evidence of nuclear degeneration in all types of cells on the first and second days after injection. As in the degenerating lymphocytes of the lymphoid organs, the chromatin of the nucleus was concentrated in discrete small opaque dense basophilic masses, and there was no sign of a nuclear membrane. In the larger myelocytes, the chromatin was massed into irregular strands, and the nuclear membrane was broken. Many of these degenerated cells remained free, but some of them had been ingested by macrophages of reticulum cell origin. A peculiar kind of degeneration was noted in the neutrophils of the SM group on the third day. The nucleus appeared as an amorphous mass of chromatin with bleblike bulbs protruding from the margin. Some of the megakaryocytes contained pyknotic nuclei, and in a few of them the nucleus had disintegrated.

An analysis of the distributions of the myeloid cells of the marrow and of the neutrophils of the circulating blood shows that there is a definite relation between the number of myelocytes and their mitotic activity and the number of neutrophils present in the blood. None of the agents seemed to cause the same degree of effect, although the character of the changes was the same. Methyl-bis (2-chloroethyl) amine caused the greatest change in that an initial decrease of myelocytes associated with continued inhibition of mitosis was accompanied by continued and marked decrease of the neutrophils of the blood and marked neutropenia of the

bone marrow. In the SM and TCA groups, where the inhibition of the mitosis of the myelocytes did not last longer than one day, there was only temporary neutropenia of the blood on the day following the inhibition of mitosis; in both of these groups, however, this was accompanied by continued decrease in the number of neutrophils of the marrow. In the ECA group, where the inhibition of mitosis was slow to appear, there was no change in the neutrophils of the blood. Thus methyl-bis (2-chloroethyl) amine seems to be the only agent which had a marked effect both on the number of neutrophils of the blood and on the myeloid elements of the marrow.

In all groups except the ECA group there were marked decreases in the numbers of nucleated erythroid cells and inhibition of mitosis of these cells for at least one day. It is thought that much of the hypoplastic appearance of the marrow was due to the retardation of the mitotic activity of these cells. Since there were many masses of mature red blood corpuscles in the sinuses, it is possible that maturation of the cells continued, but the cells were not replaced as they matured. There seems to be no doubt that anemia would eventually occur in these rats. When the mitotic activity of the erythroid cells was resumed, there was no evidence that these cells were proliferating from the endothelial cells of the sinusoids, but the islands of erythroid cells appeared to arise from erythroid cells which had remained uninjured in the intersinusoidal reticulum spaces.

COMMENT ON THE BONE MARROW

The percentage distributions of the cells of the bone marrow of the control rats were like those described by Stasney and Higgins,[48] Kindred [1] and Töppner,[49] and the distributions of cells per unit volume agreed with those given for the rat.[50] Study of the bone marrow of men gassed with sulfur mustard in World War I showed that there was aplasia in those with the greatest neutropenia of the circulating blood. The neutrophils had been swept out, and there was peripheral hyperplasia of myeloid cells indiscriminately arranged.[2a] In rabbits given injections of sulfur mustard there was aplasia four days after injection, with decrease in mitosis of the remaining cells. These degenerative changes were followed, even after a second injection of sulfur mustard, by regeneration of all types of marrow cells and increase of mitosis.[3a] In reports of recent investigations, degenerative changes of the marrow have been described as following injection of, or exposure to, sulfur mustard,[51] ethyl-bis (2-chloroethyl) amine (very slight change),[44c] methyl-bis (2-chloroethyl) amine [52] and tris (2-chloroethyl)

48. Stasney, J., and Higgins, G. M.: Anat. Rec. **63**:77, 1935.

49. Töppner, R.: Folia haemat. **66**:48, 1942.

50. Farrar, G. E., Jr.: Am. J. Physiol. **117**:662, 1936. Kindred.[1]

51. (*a*) Salter, W. T.; Bullock, T. H., and Fishman, J. B.: Unpublished data, 1943. (*b*) Cameron, G. R.: Unpublished data, 1942.

52. Cameron, G. R., and Foss, G. I.: Unpublished data, 1942. Karnofsky and others.[20] Cameron and Short.[44b] Graef and others.[44c] Crawford and Smith.[48a] Salter and others.[51a]

amine.[53] Tris (2-chloroethyl) amine was most toxic for rats when administered by gassing or by subcutaneous injections; when the agents were injected intravenously, sulfur mustard had the most toxic effect.[54] In the present investigation, in which the agents were intravenously injected, tris (2-chloroethyl) amine was found to be the most toxic, but methyl-bis (2-chloroethyl) amine had the longest depressive effect. The effects of these agents do not seem to be as damaging to the cells as roentgen radiation,[55] since there was not as much cell necrosis and phagocytosis. Mitosis of the myeloid cells is inhibited temporarily, but the number of neutrophils seems to be decreased by migration rather than by destruction. It is possible that the neutrophils that were on the way to maturity were stimulated to more rapid maturation, whereas those which were growing were inhibited. The latter view rests on the observation that young myelocytes were diminished in number in the first two days after injection and increased during the last two. Hyperemia of the marrow is characteristic, and this was also observed in mice exposed to roentgen rays.[26]

The myelocytes show reduction in number and evidence of injury after being exposed to methyl-bis (2-chloroethyl) amine.[56] They are destroyed by roentgen rays in mice [57]; they are decreased in absolute number in the rat [58] and are the cells particularly affected by roentgen rays.[59] These observations are in agreement with the initial changes observed in the myelocytes after injection of the vesicants. Mitosis, it has been concluded in the present investigation, was initially inhibited in the rats injured by injected vesicants; it has been observed by others to be inhibited as a result of injury produced with mustard,[60] with methyl-bis (2-chloroethyl) amine [29] and with roentgen rays.[61] Mature neutrophilic granulocytes, which decreased in number on the second day after injection and continued to decrease in the marrow of the poisoned rats, and which in the MCA group showed greater injury than in the other groups, have been found to react in the same manner to methyl-bis (2-chloroethyl) amine by Karnofsky and associates.[29] They decreased in rabbits after injection of sulfur mustard [3a] and in men after exposure

53. Graef and others.[44c] Short.[46a] Hanck.[46b]

54. Dougherty, T. H.; Goodman, L. S.; Gilman, A., and Dougherty, J.: Unpublished data, 1942.

55. Heineke, H.: Deutsche Ztschr. f. Chir. **78**:196, 1905. Dunlap.[17] Warren and Whipple.[43a]

56. Karnofsky and others.[29] Cameron and Short.[44b]

57. Warthin, A. S.: Internat. Clin. **4**:243, 1906.

58. Lingley, J. R.; Gall, E. A., and Hilcken, J. A.: Am. J. Path. **16**:845, 1940.

59. Ssipowsky, P. W.: Beitr. z. path. Anat. u. z. allg. Path. **94**:1, 1934.

60. Krumbhaar.[2a] Pappenheimer and Vance.[8a]

61. Mottram, J. D.: Arch. Radiol. & Electrotherap. **25**:197, 1920. Warthin.[57]

to this vesicant.[62a] They showed no change in number in mice during the first forty-eight hours after being exposed to roentgen rays but were gradually reduced in number after this time.[62]

Erythrogenic cells, which are more resistant to the effects of the vesicants than are the myeloid cells, have shown injury only after being exposed to roentgen rays for a long time.[58] The megakaryocytes, which have been shown here to exhibit some signs of injury but no reduction in number, are reported to react in the same way to methyl-bis (2-chloroethyl) amine [29]; with sulfur mustard they are reduced in number for some time after the initial injection.[58a] Following roentgen irradiation they are said to be damaged and reduced in number.[63] Eosinophils which were reduced in number in the poisoned rats during the last two days of the four day period have been observed to be injured forty-eight hours after injection with methyl-bis (2-chloroethyl) amine.[29] Thus in general the effects of the vesicants on the several stages of development of the white and red cells of the marrow are the same as those produced by roentgen rays, and except for the injuries caused by methyl-bis (2-chloroethyl) amine, are not as drastic.

In the normal rat the marrow's production of mature granulocytes has been calculated to be sufficient to supply about fifty-eight times as many granulocytes per hour as are needed by the circulating blood to maintain the average count.[1] Hence it appears that even if this source were temporarily inhibited by the vesicants, there would be no immediate neutropenia of the blood. It is possible that if the toxic action continued, neutropenia would occur. In the table showing the distributions of the neutrophils of the blood (table 1, C), it will be seen that only in the MCA group was there a trend toward neutropenia, and in this group there was more destruction of the myeloid cells of the marrow than in the other groups.

According to Dougherty and White,[21b] no extensive degeneration of the myeloid elements was seen in mice given injections of pituitary adrenocorticotropic hormone, although some degenerated lymphocytes were observed in macrophages. In view of these observations a limited quantitative study was made of the femoral marrow of adrenalectomized rats that received injections of 0.3, 0.4 and 0.5 mg. of tris (2-chloroethyl) amine per kilogram of body weight. In the marrow of these rats there was a tendency toward hyperemia and hypoplasia at the end of the first day after injection in those rats receiving the largest amount of the agent. There were no changes in the proportions of the different types of cells. Such a condition obtained in nonadrenalectomized rats on the

62. Lawrence and Tennant.[26] Warren and Whipple.[43a] Warthin.[57] Lingley and others.[58]

63. Shouse and others.[24] Heineke.[55] Ssipowsky.[59]

first day after injection of 1.0 mg. of tris (2-chloroethyl) amine per kilogram of body weight. On the second day after injection the hyperemia and the hypoplasia were characteristic of all groups. Many of the neutrophils were injured, but no injured lymphocytes were observed. There were large edematous spaces between the sinusoids. The myeloid cells were scattered, and in them mitosis seemed to be as in the normal myelocytes. A few macrophages were observed. The most notable characteristic was the practical absence of erythroblasts and normoblasts. The megakaryocytes were prominent, and their nuclei were swollen. The percentages of the neutrophils were significantly less than in the control group (starved adrenalectomized rats), and there was increase in the relative proportions of myeloblasts and myelocytes. These conditions continued throughout the four day period in the rats which survived. These observations supplement the data obtained from the study of the lymphoid organs of these adrenalectomized rats in support of the view that the agent acts directly on the cell and its effect is not mediated by the adrenal gland.

SUMMARY

In young adult albino rats given single intravenous injections of lethal doses of sulfur mustard (bis [2-chloroethyl] sulfide) and the nitrogen mustards (ethyl-bis [2-chloroethyl] amine, methyl-bis [2-chloroethyl] amine and tris [2-chloroethyl] amine) the following major changes occurred in the circulating blood and the hemopoietic organs: (1) lymphopenia in all groups; (2) lymphopenia and neutropenia in the group given methyl-bis(2-chloroethyl) amine; (3) reduction of the weight and the amount of the lymphoid tissue of the thymus, the cervical lymph nodes and the spleen, and (4) hypoplasia and hyperemia of the femoral marrow.

Tris (2-chloroethyl) amine caused the greatest lymphopenia and had the most degenerative effect on the lymphocytes of the thymus, the lymph nodes and the spleen. Next in order of toxicity were the ethyl-bis and methyl-bis (2-chloroethyl) amines, which produced almost identical degenerative effects on these organs. Sulfur mustard was least effective initially but caused terminal changes of the same degree as those produced by the nitrogen mustards.

In the lymphoid organs the lymphoid tissue shrinks in volume because of the destruction of lymphocytes, the inhibition of mitosis of the medium-sized lymphocytes and the migration of lymphocytes which go into the blood stream. The reticulum cells of the lymphoid organs are not injured directly by the agents. These cells as fixed phagocytes ingest and digest the degenerated lymphocytes. It is believed that many of them perish from the exhaustion resulting from their unusual phagocytic activity. Regeneration of the lymphoid tissue

begins on the third day after injection, in general, and is characterized by increase of number and mitotic activity of the medium-sized lymphocytes. It is believed that these cells arise from small lymphocytes which have escaped the toxic action of the agents.

In adrenalectomized rats given intravenous injections of tris (2-chloroethyl) amine the same degenerative changes occurred as were observed in the nonadrenalectomized rats. Hence it is believed that the damage done to the lymphocytes in the lymphoid organs of the vesicant-injured rats is caused by direct action of the agents. Further evidence for the theory that the nitrogen mustards act directly on the lymphocytes accrues from the in vitro study of fragments of thymus suspended in plasma and tris (2-chloroethyl) amine. In these preparations the lymphocytes showed the same karyorrhectic changes which characterized them in the lymphoid organs of rats given injections of vesicants.

There is a general direct correlation between the decrease of the number of lymphocytes of the blood and the degeneration of the lymphoid organs. However, it is thought that the initial loss of lymphocytes of the blood stream results because the lymphocytes migrate through the degenerated epithelium of the intestine into the lumen.

The bone marrow reacted more slowly to the vesicants than did the lymphoid organs, but it became hypoplastic and hyperemic. There was some destruction of cells, particularly of the mature granulocytes. The most marked effects were the decrease of the number of mature granulocytes and the inhibition of the mitosis of myelocytes lasting one day. Regeneration of myelocytes began on the third day after injection, but their maturation was apparently inhibited except in the sulfur mustard group. Methyl-bis (2-chloroethyl) amine had a more depressing effect on the myeloid cell population than did the other agents. Ethyl-bis (2-chloroethyl) amine had a slight effect.

The nucleated erythroid cells of the marrow decreased markedly in number, and their mitosis was inhibited. Regeneration began in these cells on the third day, and the cells which were dividing appeared to arise from erythroblasts which had escaped injury by the agents.

The megakaryocytes seemed to suffer the least change of any of the hemopoietic cells. No phagocytes containing great masses of degenerated cells were seen in the bone marrow, and in this respect there was a difference between the degenerative changes of the lymphoid organs and those of the marrow.

The degenerative changes of the hemopoietic organs as a whole resembled more closely those produced by roentgen rays than those caused by any other agents.

ACUTE AND CHRONIC TOXICITY OF METHYL CHLORIDE

IV. Histopathologic Observations

ROBERT C. DUNN, M.D.
AND
WILLIE W. SMITH
BETHESDA, MD.

PRECEDING reports deal with the properties of methyl chloride, the method of exposure, and the symptoms [1] and the mortality resulting in animals exposed to concentrations ranging from 4,000 to 300 parts per million,[2] and with the results of hematologic and biochemical studies.[3] Histopathologic material derived from these experiments will be described in this communication.

LITERATURE

Autopsy findings in fatal human methyl chloride poisoning have been reported by Schwarz,[4] Kegel, McNally and Pope [5] and McNally.[6] Sayers and his associates [7] have made an extensive study of guinea pigs, and further observations on this species have been reported by White and Sommers [8] and Schwarz.[4] Schwarz has also reported findings in mice and rabbits, and Montmartini [9] findings in dogs. The principal abnormalities reported by these authors were: congestion, edema and hemorrhage in various organs, particularly the lungs; fatty changes in

From the Pathology Laboratory (Robert C. Dunn) and the Industrial Hygiene Research Laboratory (Willie W. Smith), National Institute of Health, United States Public Health Service.

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the liver; degenerative changes in the kidneys and the liver, and degeneration of anterior horn cells.[4] No mention was made of necrosis of renal cortical tubules occurring in mice and rats, or of hemoglobinuria and associated conditions in mice, which were observed with great frequency in the present study.

METHODS

Seven species of animals were exposed to concentrations of methyl chloride ranging from 4,000 to 500 p.p.m. for six hours a day six days a week until death. The eighth species studied was exposed to 11,000 p.p.m. for two hours a day on two consecutive days.

Organs were fixed in 4 per cent solution of formaldehyde buffered to p_H 7. Paraffin sections were stained routinely with eosin–polychrome methylene blue, prussian blue (used as a reagent for the detection of iron) and the hemoglobin-collagen stain.[10] Frozen sections were stained by the oil red O–isopropyl alcohol technic.[11] Brain and spinal cord were fixed in Orth's solution.

OBSERVATIONS

Mice.—Mice of three strains, 3 to 4 months old and equally distributed as to sex, were exposed to 2,000 p.p.m. Except for 5 mice of one strain (C_3H), the animals died during the week of exposures. Litter mate controls showed no significant lesions.

The evidences of injury of the kidneys, fat, necrosis, hemoglobin globules and hemoglobin casts are shown in the accompanying table. The epithelium of scattered convoluted tubules showed various degenerative changes, grading into

Renal Changes Observed in Mice Exposed to Methyl Chloride 2,000 Parts per Million

	Swiss Strain				Strain A				Strain C_3H			
		Degree of Change				Degree of Change				Degree of Change		
	Mice	Slight	Moderate	Marked	Mice	Slight	Moderate	Marked	Mice	Slight	Moderate	Marked
Fat	9	2	2	0	15	3	3	0	15	2	6	0
Necrosis	25	7	11	0	15	4	9	1	15	5	6	0
Hemoglobin globules	25	0	1	2	15	2	7	6	15	0	4	0
Hemoglobin casts	25	1	3	1	15	4	5	0	15	2	3	6

necrosis and fibrocellular replacement scars infiltrated by a few to moderate numbers of polymorphonuclear neutrophils. In the earlier stages of this process epithelial cells of a few to many convoluted tubules and occasionally the thick portion of Henle's loop displayed pyknotic nuclei and fibrillar basophilic cytoplasm. In the cortex of some kidneys in which necrosis was present there were radially arranged elongated areas of regeneration in which tubules were lined by large basophilic epithelial cells, which occasionally showed mitosis. Hemoglobin globules were often found in the cytoplasm of cells lining the convoluted tubules. In addition to the hemoglobin casts there were occasionally small numbers of eosinophilic hyaline or basophilic fibrillar casts.

10. Dunn, R. C., and Thompson, E. C.: Arch. Path. **39**:49, 1945.
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There were slight to moderate fatty changes in the kidneys of about half of the animals in each group, the fat finely globular and predominantly in the epithelial cells of the convoluted tubules. Occasionally a kidney showed a few altered glomeruli, the changes consisting of shrunken or very cellular glomerular tufts, hyaline masses in the lumens of capillaries of tufts, hyalinized capillary walls, or basophilic fibrillar debris in the capsular space. In many mice, at autopsy the urine of the bladder was reddish, gave a strongly positive reaction in the benzidine test for hemoglobin and contained many reddish granular casts.

In all but a few mice the hepatic cells in the centrolobular areas of the liver showed a moderate degree of fatty metamorphosis. There was moderately extensive necrosis in the centrolobular areas in 4 of these mice. Congestion was the only pulmonary change noted with any regularity, though a slight degree of edema was noted rarely, and hemorrhage in occasional alveoli. Occasionally myocarditis was seen but was probably infectious.

Rats.—In general the pathologic changes seen in the kidneys of rats were similar to those seen in the kidneys of mice, with the exception of the findings associated with hemoglobinuria. In all of a group of 10 rats dying after the fourth or fifth exposure to 4,000 p.p.m., and in 8 of the 16 rats dying after the fifth exposure to 3,000 p.p.m., the kidneys showed few to moderately numerous necrotic convoluted tubules. Half of a group of 12 rats 4 months old exposed to 2,000 p.p.m. for two weeks died and showed the cortical necrosis described; those that were killed, and the 11 litter mate controls, showed none. Occasionally small amounts of fat appeared in the epithelial cells of the convoluted tubules.

Small to moderate amounts of fat were frequently seen in hepatic cells, and centrolobular necrosis was occasionally noted in the liver. In the lungs congestion and, rarely, a slight degree of edema were the only pathologic changes which were not also encountered in unexposed rats. Occasionally myocarditis, apparently infectious, was noted. Rats exposed for six to nine months to 1,000 and 500 p.p.m. showed no significant changes.

Guinea Pigs.—Unlike mice and rats, guinea pigs showed neither renal necrosis nor hemoglobin deposits. However, fatty metamorphosis of moderate to marked degree was noted in both kidney and liver in 7 of 14 guinea pigs dying after one to three daily exposures to 3,000 or 2,000 p.p.m. These fatty changes were observed chiefly in the epithelial cells lining the convoluted tubules and the thick portion of Henle's loop and in hepatic cells in the centrolobular areas. Of 31 animals exposed to 1,000 p.p.m., fatty metamorphosis was present to a slight degree in 12 and to a moderate to marked degree in 4 others. In the same animals the liver showed moderate to marked amounts of fat.

In the lungs of guinea pigs dying within the first few days of being exposed to 3,000 or 2,000 p.p.m., marked congestion and edema were usually observed, while after exposures to lower concentrations of methyl chloride there was little change.

Occasional animals showed basophilic renal casts, traces of hemosiderin in the hepatic and Kupffer cells, and myocarditis. The adrenal glands of 4 animals showed no change. Guinea pigs surviving nine months of exposure to 500 p.p.m. showed no pathologic changes.

Dogs.—Fatty changes of the liver and the kidney and renal necrosis were minimal or absent. In 1 of 6 dogs exposed to 3,000 p.p.m. the urine was reddish brown and strongly reactive with benzidine, and there were a few small granular hemoglobin casts in the kidney. Hemosiderin was found in small amounts in renal, hepatic and Kupffer cells, and in large amounts in the spleen. In other dogs of

this group there was slight to marked splenic hemosiderosis, but hemosiderin was not found in the other organs and hemoglobin casts and hemoglobinuria were absent, although hemoglobinuria was occasionally noted in other dogs just before death.[3] In dogs exposed to 2,000, 1,000 and 500 p.p.m., hemosiderin was usually found in moderate to large amounts in the spleen and often in moderate amounts in the Kupffer cells of the liver and in the epithelium of the convoluted tubules of the kidney.

The lungs of dogs exposed to 3,000 p.p.m. often showed marked congestion, and in an occasional dog there were small amounts of blood in scattered alveoli or a moderate number of hemosiderin-laden macrophages in the alveolar septums. Adrenal glands, stomach, pancreas, ovaries and heart appeared unchanged.

The brains and spinal cords of 5 pups, in all of which had developed severe neuromuscular symptoms[1] after exposures ranging from one week at 2,000 p.p.m. to thirty weeks at 500 p.p.m., were examined by Dr. R. D. Lillie who noted gross and microscopic lesions which appeared to be of an infectious nature and from which possible toxic morphologic changes could not be dissociated. Similar lesions were observed in an unexposed litter mate.

Rabbits.—Most rabbits exposed to 4,000 p.p.m. methyl chloride died after the second week of exposures, and those exposed to 2,000 p.p.m., after four to five weeks. Pulmonary congestion, often accompanied by edema, was frequently seen in rabbits dying in the first week but not in those dying thereafter. Many of the rabbits showed pneumonia, possibly as a secondary effect of the exposures. The only other changes of significance were found in a rabbit dying after twenty-seven weeks of exposures to 500 p.p.m. and included moderate fatty metamorphosis of the liver, the kidney and the heart, and extensive coagulation necrosis of the centrolobular areas of the liver. Three rabbits dying after exposures to 4,000 p.p.m. and 2 others killed after two weeks of exposures to 3,000 p.p.m. showed no significant changes. Chronic myocarditis in 1 animal proved to be an extension from the adjacent suppurating lung. No pathologic changes were observed in brain, spinal cord or sciatic nerve.

Cats.—In a cat which became moribund and was killed in the third week of being exposed to 2,000 p.p.m., the lungs were moderately congested, macrophages in the alveolar septums contained moderate amounts of hemosiderin, and hepatic cells contained many fine to coarse fat globules diffusely distributed. There were similar findings in 2 other cats that died in the fifth week of exposures.

Monkeys.—A single monkey, dying after sixteen weeks of exposures to 500 p.p.m., was examined for microscopic changes. Other than a fairly marked hemosiderosis of spleen and Kupffer cells, with traces of hemosiderin in hepatic cells, all organs examined (lungs, liver, kidneys, heart and spleen) appeared to be normal.

The brain of a monkey that died in the second week of exposures to 2,000 p.p.m., and the brains and the spinal cords of 2 others that were exposed intermittently to that concentration over periods of four and nine weeks, respectively, were examined by Dr. R. D. Lillie, who observed no pathologic changes distinctly attributable to methyl chloride.

Goats.—Grossly, the only pathologic changes in 2 goats exposed for two hours a day on two consecutive days to 11,000 p.p.m. were petechial hemorrhages in the small intestine and the adjacent mesentery. In the one goat the kidney had a mottled and hyperemic appearance and the lungs were hyperemic with a small amount of foamy mucus in the bronchi; in the other the lungs were very hyperemic

and appeared to be slightly edematous. These observations were confirmed microscopically, with the addition that moderate numbers of fat globules, mostly fine, were diffusely distributed in the hepatic cells.

SUMMARY

On the basis of a study of several hundred animals, the majority mice, rats and guinea pigs, with fewer dogs, rabbits, cats, monkeys and goats, the only morphologic changes that appear to be a direct result of inhalation of methyl chloride are variable degrees of necrosis of the convoluted tubules of the kidneys in mice and rats, renal changes associated with hemoglobinuria in mice and occasional dogs, and a fairly constant but low to moderate amount of fatty metamorphosis of the liver and kidneys in the smaller species of animals studied. Clinically, pulmonary edema was noted frequently and appeared to be a direct result of the irritation due to inhalation of methyl chloride. Although focal myocardial changes and pneumonia were occasionally noted, they were often closely associated and did not appear to result directly from the inhalation of methyl chloride.

HISTOLOGIC COMPARISON OF THE BRAINS OF VITAMIN A–DEFICIENT AND VITAMIN E–DEFICIENT CHICKS

F. B. ADAMSTONE, Ph.D.
URBANA, ILL.

AS POINTED out by Moore,[1] considerable evidence has been accumulated indicating a definite synergistic relation between the vitamin A and the vitamin E of a ration. This is shown by the defective storage of vitamin A in animals deficient in vitamin E and also by the effect of vitamin E on the growth response of animals receiving vitamin A. It is possible, therefore, that certain reactions in experimental animals which have been attributed to vitamin E deficiency may also be related to an unsuspected vitamin A deficiency. A case in point is encephalomalacia occurring in the chick. Under conditions of vitamin E deficiency a characteristic pathologic syndrome develops which is marked by a typical histologic picture of brain degeneration (Pappenheimer and Goettsch [2]; Adamstone [3]). Extensive studies of other phases of this condition have been made by Pappenheimer, Goettsch and Jungherr,[4] as well as by Dam,[5] Dam and Glavind,[6] Bird [7] and others. Patrick and Morgan [8] presented evidence that vitamin A deficiency may arise spontaneously in vitamin E–deficient diets which have produced encephalomalacia and suggested that so-called field encephalomalacia may be in reality a vitamin A deficiency disease. Moreover, in experiments in which young chicks were reared on diets deficient in vitamins A and E, respectively, the reactions produced were similar in both cases. Hence, since diagnosis is difficult, a careful histologic study of the two conditions is highly desirable in order to determine whether they can be distinguished with certainty by histologic methods and also whether the findings support the suggested synergistic relation between vitamins A and E. The present report gives the results of a study carried out with this object in view.

From the University of Illinois.

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MATERIALS AND METHODS

For the purposes of these investigations groups of newly hatched chicks were reared in wire-floored brooder pens on rations designed to produce vitamin A and vitamin E deficiency. The following diets were employed:

(1) Basal Ration—Control

Ground yellow corn	46
Wheat bran	13
Wheat middlings	13
Alfalfa meal	5
Meat scrap	10
Dried skim milk	10
Salt	1
Cod liver oil	2
	100

(2) Vitamin A–Deficient Ration

Ground white corn	52
Wheat bran	13
Wheat middlings	13
Meat scrap	10
Dried skim milk	10
Salt	1
	99
Cod liver oil	0 to 1 per cent

(3) Iron-Treated Vitamin E–Deficient Ration: Ration 1 treated with ferric chloride dissolved in ether (Adamstone [3]).

(4) Ration Deficient in Vitamins A and E: Ration 2 rendered E deficient by treatment with ferric chloride.

(5) Purified Ration—E Deficient. This was made up as follows:

Casein	250 Gm.	Salts	45 Gm.
Dextrin	500 Gm.	L. Cystine	3 Gm.
Sucrose	100 Gm.		
Lard	102 Gm.		1,000

Supplements per Thousand Grams of Ration 5

Thiamine hydrochloride	5 mg.	Choline chloride	1,000 mg.
Riboflavin	10 mg.	Biotin	2 mg.
Pyridoxine	5 mg.	Vitamin A	50 U.S.P. units
Nicotinic acid	100 mg	Vitamin D	5 U.S.P. units
Calcium pantothenate	15 mg.	Vitamin K	5 mg.
p-aminobenzoic acid	150 mg.	Yeast	5 per cent
Inositol	400 mg.	Gelatin	10 per cent

Summary of Experiments

Lot	Vitamin Deficiency	Supplement *	Chicks	Chicks Showing Imbalance	Chicks Showing Microscopic A-Deficiency Lesions	Chicks Showing E-Deficiency Lesions of Brain: Gross Lesions	Chicks Showing E-Deficiency Lesions of Brain: Microscopic Lesions
1	Control	2% C.L.O.	15	0	..	..	..
2	A	U.V.L.	20	20	10	..	..
3	A	¼% C.L.O.	15	15	10	..	..
4	A	½% C.L.O.	15	0	..	..	..
5	A	1% C.L.O.	15	0	..	..	..
6	A, E	2% C.L.O.	20	20	10	..	2
7	E	2% C.L.O.	20	12	11	12	12
8	E	0.4% H.L.O.	18	7	5	7	7
9	E	Synthetic	20	3	..	3	3

* C.L.O. means cod liver oil; H.L.O., halibut liver oil; U.V.L., ultraviolet rays, given twenty minutes daily.

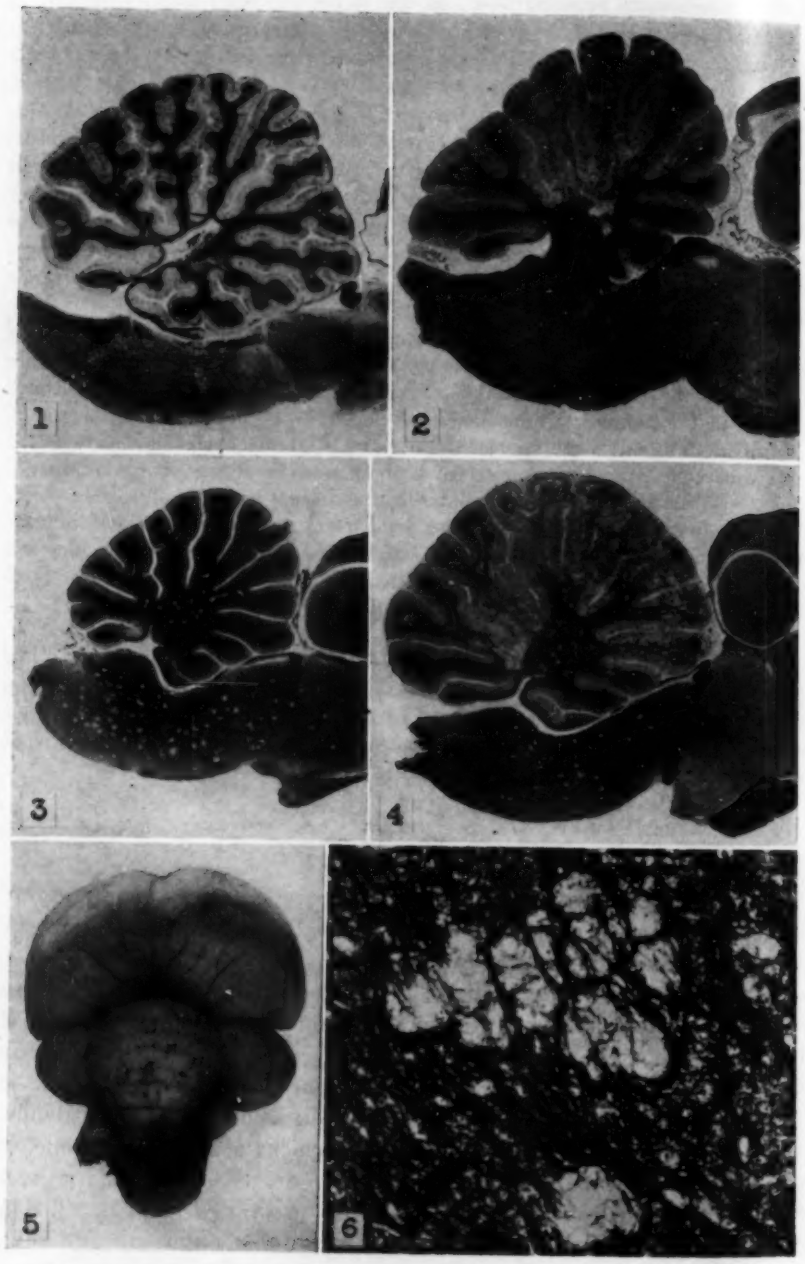
The experimental program based on use of the described diets is outlined in the accompanying table. A brief explanation of the plan of the experiment is necessary. In lots 2, 3, 4 and 5 the vitamin A–deficient diet (ration 2) was used. In lot 2 the chicks were exposed to ultraviolet rays for twenty minutes daily in order to protect them against vitamin D deficiency. In lots 3, 4 and 5, different supplements of cod liver oil were used in order to find the level at which the birds were completely protected from A deficiency. In lot 6 the vitamin A–deficient ration 2 was treated with ferric chloride dissolved in ether in order to superimpose a vitamin E deficiency as well. For lots 7 and 8 the iron-treated vitamin E–deficient ration was employed, but halibut liver oil was used in lot 8 in place of cod liver oil. In lot 9 the birds were fed the purified ration supplemented with the synthetic vitamins listed.

As the experiment progressed the chicks were watched carefully for signs of imbalance so that a sufficient number might be killed before death in order to secure well fixed brain material. From the data given in the table it will be seen that symptoms of imbalance developed in all birds in lots 2 and 3. A total of 20 brains were secured from them. All the chicks in lots 4 and 5 were apparently completely protected and showed no signs of incoordination. In lot 6 imbalance developed in all birds, and from these birds 10 brains were obtained. In lots 7 and 8 there were some chicks showing imbalance in spite of the rather high amounts of cod and halibut liver oil with which the diet was supplemented in those experiments. Finally, in lot 9 there were a few more birds showing incoordination.

The brain of each affected chick was quickly removed at autopsy after decapitation and fixed in a 4 per cent solution of formaldehyde or in 95 per cent alcohol. Later it was sectioned at 6 to 10 microns and the slides were stained with Heidenhain's iron-alum-hematoxylin or with cresyl violet. For this study the brains of the E–deficient birds were supplemented with a great deal of material secured earlier in other similar experiments.

CLINICAL SYMPTOMS OF VITAMIN A AND VITAMIN E DEFICIENCY IN THE CHICK

Both A and E deficiency in the chick are characterized by severe locomotor disturbances having their origin in the central nervous system. In both there are definite incoordination and imbalance, making the two conditions difficult to distinguish. In general the following differences may be observed, but they are not sufficiently cleancut to be reliable. Chicks deficient in vitamin A are usually considerably smaller than those deficient in vitamin E if they are of comparable age, and in A-deficient birds the characteristic incoordination develops gradually. When the condition is well established the chicks usually stand hunched and unsteady, and if disturbed, they walk in an uncertain and irregular manner. Eventually the affected birds are prostrated and lie still, but if set on their feet, they fall quickly, often without attempting to walk. In E-deficient birds the incoordination usually appears with great suddenness, and the larger and more rapidly growing birds are often the first to be affected. These birds are also inclined to mope, balancing themselves somewhat unsteadily. If disturbed, they usually run and



FIGURES 1 TO 6

(See legends on opposite page)

stagger rapidly across the pen. When prostrated they lie with legs outstretched, wings spread out and head retracted. Often, when disturbed, they execute "bicycling movements" with their legs. A coarse tremor is evident throughout the body—this reaction being more violent than in A-deficient chicks. Obviously it is difficult to distinguish vitamin A and vitamin E deficiency in chicks on the basis of the clinical symptoms described, and one must rely on a careful histologic examination of the brains.

GROSS APPEARANCE OF BRAINS

In contrast to brains of vitamin E–deficient chicks, the brains of vitamin A–deficient birds show no visible gross lesions of any sort when cut longitudinally. Their brains are, however, smaller than those of normal birds of the same age because of the slower growth (compare figs. 1 and 3). The brains of E-deficient birds show distinct regions of hemorrhage or areas of greenish yellow discoloration (fig. 5). These lesions are sometimes hidden because the surface tissues are unaffected, but they are readily recognized in longitudinal sections. Another characteristic feature of E-deficient brains is the common occurrence of edema, which may be so pronounced that fluid escapes in considerable quantity when the tissues of the brain are cut.

Although the brains of vitamin A–deficient chicks showed no conspicuous gross lesions, examination of longitudinal sections with a hand lens revealed numerous pinpoint areas of degeneration in certain areas (fig. 3). These were most frequently seen in the brain stem, the optic chiasma, the base of the cerebellum and, in a few rare cases, the cerebral hemispheres.

Birds receiving vitamin E–deficient diets showed typical degenerate areas characteristic of nutritional encephalomalacia in the cerebellum (figs. 2, 4 and 5) or, more rarely, in the cerebral hemispheres. Occasionally, small pinpoint lesions similar to those found uniformly in A-deficient brains were to be seen in supposedly E-deficient brains (fig. 4). It would appear, therefore, that an incipient condition of A deficiency was present in birds in which such lesions occurred. This

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EXPLANATION OF FIGURES 1 TO 6

Fig. 1.—Longitudinal section of the cerebellum of a normal chick.

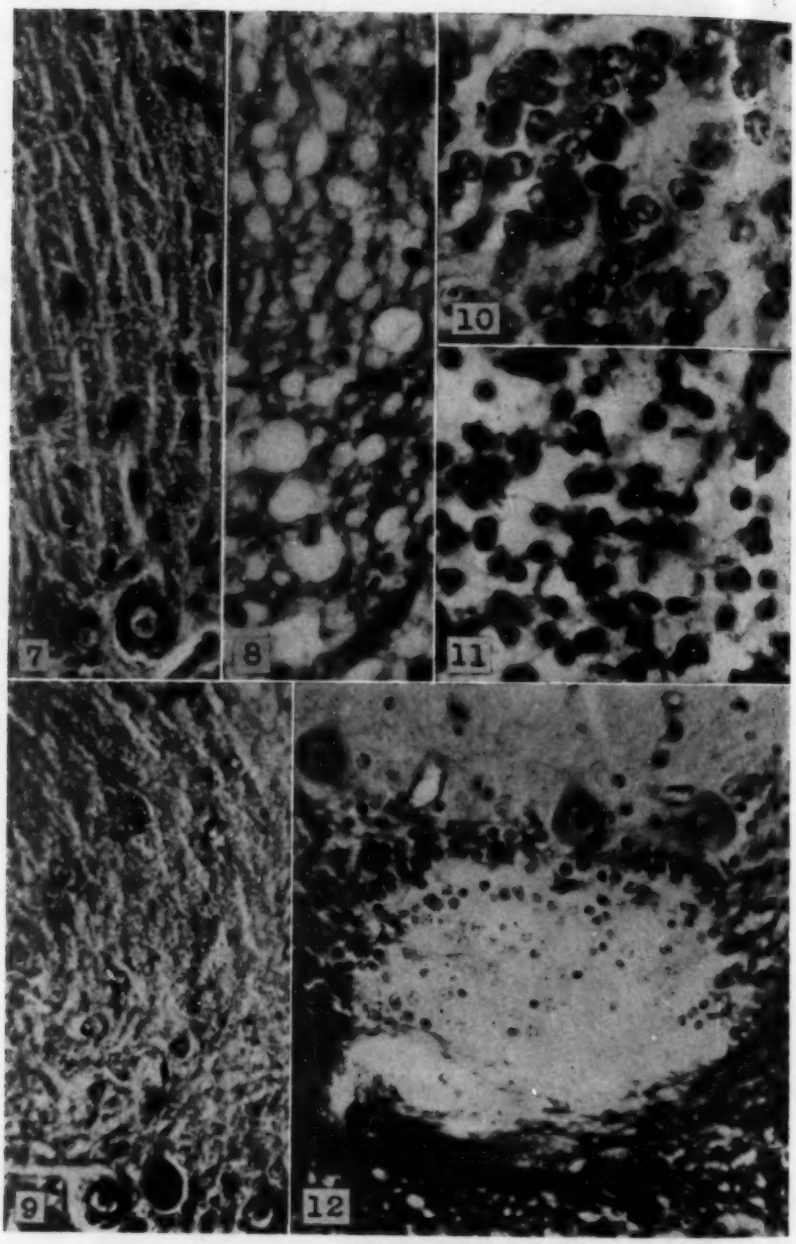
Fig. 2.—Longitudinal section of the cerebellum of a vitamin E–deficient chick.

Fig. 3.—Longitudinal section of the cerebellum of a vitamin A–deficient chick showing the characteristic pinpoint lesions.

Fig. 4.—Longitudinal section of the cerebellum of a vitamin E–deficient chick showing the characteristic A-deficiency lesions.

Fig. 5.—Brain of vitamin E–deficient chick showing a hemorrhage at the base of the cerebellum.

Fig. 6.—Pinpoint lesions in the brain stem of a vitamin A–deficient chick under higher magnification.



FIGURES 7 TO 12

(See legends on opposite page)

contrasts strikingly with the A-deficient brains, all of which except two were free from E-deficiency lesions. In the two excepted (lot 6) E deficiency had been experimentally superimposed on A deficiency.

DETAILED HISTOLOGIC COMPARISONS

It is proposed now to describe in detail the lesions in various parts of the brains of the experimental chicks so that they may be readily compared. The lesions are so distinctive that a carefully prepared series of photomicrographs is presented to show the contrast between similar areas of normal, vitamin E–deficient and vitamin A–deficient brains.

Vitamin E-Deficient Brains.—In E-deficient brains degeneration is usually confined to the cerebellum, although similar changes may occur more rarely in the cerebrum. The general histologic picture seen in the cerebellum may be outlined briefly as follows:

(*a*) Outer molecular layer: disorganization of the dendrites of Purkinje cells, producing first a reticulated appearance and finally complete disorganization (fig. 8).

(*b*) Pyknosis and degeneration of the cells of the Purkinje layer (figs. 8 and 20).

(*c*) Pyknosis and degeneration of cells of the inner granular layer (fig. 11).

(*d*) Disorganization of the fibers of the medullary layer of the convolutions (fig. 14).

(*e*) Extensive hemorrhage and hyaline thromboses occurring in all layers of the cerebellum. These are seen in the degenerate areas even at low magnifications (fig. 2).

The picture of degeneration described is typical of ischemic necrosis, and it is usually sufficient to enable the observer to diagnose nutritional encephalomalacia caused by vitamin E deficiency. In the present study another detail has come to light which makes the distinction even more certain. In the fibers of the medulla of the cerebellum where extensive disorganization has occurred there are often found areas which have a peculiar reticular appearance. At higher magnifications, however, this is seen to be caused by blister-like swellings on the individual nerve fibers. These vary greatly in size and frequency of occurrence, some fibers

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EXPLANATION OF FIGURES 7 TO 12

Fig. 7.—Outer molecular layer of the cerebellum of a normal chick.

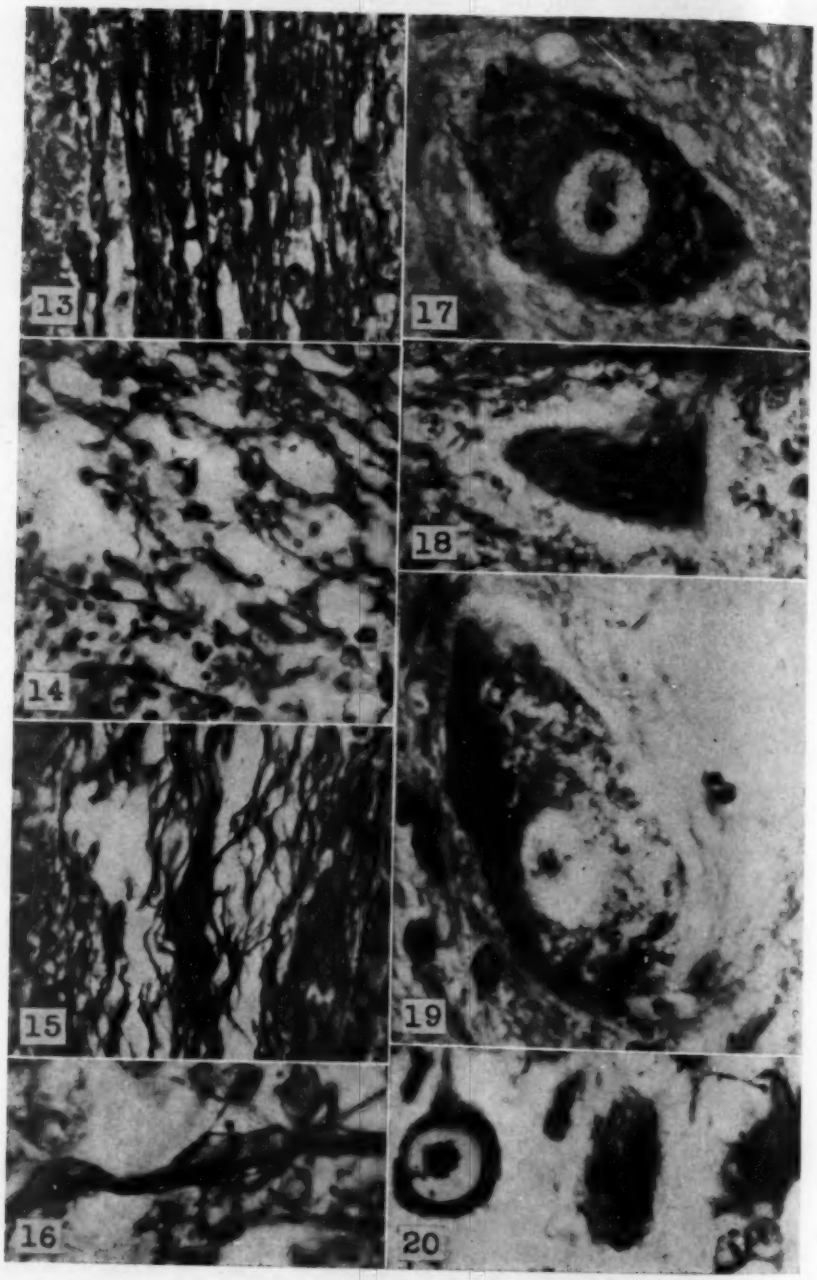
Fig. 8.—Outer molecular layer of the cerebellum of a vitamin E–deficient chick. The disintegration of dendrites produces a typical reticular appearance.

Fig. 9.—Outer molecular layer of the cerebellum of a vitamin A–deficient chick. The dendrites show little abnormality.

Fig. 10.—Cells of the inner granular layer of a normal brain, showing clear vesicular nuclei.

Fig. 11.—Pyknosis of nuclei of cells of the inner granular layer of the cerebellum of a vitamin E–deficient chick.

Fig. 12.—Degenerate area in the inner granular layer of the cerebellum of a vitamin A–deficient chick. Nuclei and cytoplasm have lost their chromaticity, and there is some loss of chromaticity in Purkinje cells at the right.



FIGURES 13 TO 20

(See legends on opposite page)

having few and others appearing almost moniliform (figs. 14 and 16). Examined under the oil immersion objective the swellings appear to be formed by the lifting of a delicate sheath (possibly an axolemma) from the surface of the nerve fiber. This could conceivably be caused by fluid accumulating within the fiber where the vesicles occur. Frequently a series of irregular holes appear to be present in the swollen sheath as if it had burst under pressure. The occurrence of this sheath and of the blister-like vesicles has been found uniformly in E-deficient brains, both those from birds receiving the iron-treated ration and those from birds receiving the purified diet. They were also found in material prepared several years ago. It may be remarked that these structures are present in material fixed and stained with a variety of technics and hence cannot be regarded as artefacts.

The degenerative changes of the cerebrum were similar to those of the cerebellum as regards both the gross and the minute changes. In the brain stem shrinkage and pyknosis of neurons occurred with considerable frequency, but many specimens also showed pinpoint lesions in both the brain stem and the optic chiasma similar to those of A-deficient birds.

Vitamin A-Deficient Brains.—The general appearance of the pinpoint lesions found in brains of vitamin A-deficient chicks is shown in figure 6. Here the lesions appear as conspicuous lighter areas against the more uniform dark background of apparently normal nerve fibers. Under higher magnifications the centers of these areas usually are seen to contain faintly staining nerve cells, and, as shown in figure 6, the process of disintegration appears to spread outward in all directions from these centers. Often the margins of these areas are marked off by an intense staining reaction. It also appears that some of the larger nerve fibers pass through such areas seemingly unaffected by the process of degeneration. Since the cerebellum of vitamin E-deficient chicks is so strikingly affected, as shown in the foregoing section, a detailed description of the cerebellum of the A-deficient chick is given now for purposes of comparison:

(*a*) Outer molecular layer: As shown in figure 9, the outer dendritic layer of the cerebellum is not greatly affected and by comparison with the normal control (fig. 7) appears to be nearly or quite normal.

(*b*) Purkinje cell layer: No clear evidence of abnormality was discovered in this layer in any of the A-deficient material (fig. 9), although in the lesion

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EXPLANATION OF FIGURES 13 TO 20

Fig. 13.—Fibers of the medulla of the cerebellum of a normal chick.

Fig. 14.—Disintegration of fibers of the medulla of the cerebellum of a vitamin E-deficient chick. Note the blister-like swellings on the fibers.

Fig. 15.—Medulla of the cerebellum of a vitamin A-deficient chick. Note the slight rarefaction of fibers.

Fig. 16.—Single fiber from the medulla of a vitamin E-deficient chick. Note the blister-like swellings and apparent holes in the raised membrane.

Fig. 17.—Neuron from a normal brain showing typical Nissl substance and large vesicular nucleus.

Fig. 18.—Neuron from the brain stem of a vitamin E-deficient chick. Note the general shrinkage and pyknosis affecting Nissl bodies and nucleus.

Fig. 19.—Neuron from the brain stem of a vitamin A-deficient chick. The neuron borders on a pinpoint lesion and shows characteristic disintegration of the nerve fiber and of the Nissl substance in the portion of the cell within the lesion.

Fig. 20.—Pyknosis and degeneration of Purkinje cells of the cerebellum of a vitamin E-deficient chick. Compare with figure 18.

shown in figure 12 there is a slight reduction of stainability, suggesting an ultimate loss of chromaticity.

(*c*) Inner granular layer: In rare cases conspicuous lesions occurred in this area (fig. 12). They resembled the pinpoint areas of rarefaction seen in the brain stem. The cells had lost their chromaticity, and their nuclei had become clear and vesicular.

(*d*) Medulla: The nerve fibers show little indication of disorganization beyond the fact that there is a definite thinning out in some regions. Iron-hematoxylin preparations show that this is probably due to demyelination of the fibers (fig. 15 vs. fig. 13).

Degeneration of Neurons in the Brain Stem: Degeneration of the larger neurons in the brain stem is the most striking reaction in A–deficient chicks, and the process of disintegration is probably similar to that which takes place in all other nerve cells that are affected (fig. 19). The cell shown in the illustration lies at the edge of an area of rarefaction, and in it the Nissl substance is undergoing degeneration where it lies in contact with the rarefied area. The Nissl granules have shrunk into thin threads and flakes which stain lightly even though they are still coarse and heavily stainable on the other side of the cell. The nucleus remains clear and vesicular but its chromatin shows a distinct loss of chromaticity as compared with the normal cell (fig. 17).

Degenerate Areas in the Cerebrum and the Optic Chiasma: Rarefied areas indicative of cellular degeneration occurred in the cerebrum in a few A–deficient birds but their location was variable. In the optic chiasma, on the other hand, lesions occurred consistently throughout the whole group (fig. 3), and it may be suspected, therefore, that some visual derangement may have been present in the A–deficient chicks.

COMPARISON OF OTHER STUDIES ON VITAMIN A AND VITAMIN E DEFICIENCY

It seems desirable to compare the histologic observations described herein with those reported by other workers who have investigated the effects of vitamin A deficiency on the nervous system. Many of these studies have been concerned with degenerative changes occurring in nerve fibers. Steenbock, Nelson and Hart [9] and Hughes, Lienhardt and Aubel [10] noted myelin degeneration in the spinal cord and in peripheral nerves in vitamin A deficiency in swine. They also noted similar changes in cattle and fowl. Mellanby reported degenerative changes occurring in the spinal cord of A–deficient dogs [11] and degeneration of neurons in the gasserian ganglion.[12] Seifried,[13] working with chicks which had been given an A–deficient diet fifteen to eighteen days after hatching, encountered incoordination and nervous disorders at four months. Lesions were found in the brain and the spinal cord involving degeneration of ganglion cells of the cerebral cortex, the dentate nucleus of the medulla and ganglion cells of the anterior horn of the spinal

9. Steenbock, H.; Nelson, E. M., and Hart, E. B.: Am. J. Physiol. **54**:14, 1921.

10. Hughes, J. S.; Lienhardt, H. F., and Aubel, C. E.: Technical Bulletin 23, Kansas State Agricultural Experiment Station, 1928.

11. Mellanby, E.: Brain **54**:247, 1931.

12. Mellanby, E.: J. Path. & Bact. **38**:391, 1934.

13. Seifried, O., cited by Robertson, E. C.: Am. J. M. Sc. **192**:409, 1936.

cord. Demyelination was noted in the spinal cord and in peripheral nerves. Zimmerman [14] observed vacuolation, swelling and chromatolysis in Nissl preparations of nerve cells of the spinal cords of rats fed an A-deficient diet. Radhahrishna Rao [15] studied neurons of the gasserian ganglion in rats, rabbits and fowls and found disorganization of the Nissl substance of these cells.

In all instances cited in the foregoing paragraph in which nerve cells have been studied, the degenerative changes observed appear similar to those encountered in the present study. By contrast, however, other workers have failed to note nervous disorders or to find lesions in nerve tissues of animals fed vitamin A-deficient diets. This is true of Suzman, Müller and Ungley,[16] working with adult dogs, of Grinker and Kandel,[17] working with monkeys, and of Swank and Davenport,[18] observing rats and guinea pigs. An explanation of the discrepancy between the observations of these authors and those cited in the foregoing paragraph may possibly lie in the fact that the most striking evidences of deficiency diseases are produced in young animals whereas much of this work giving negative results was done with older animals.

Another study of direct interest is that of Duncan,[19] who compared the nerves of rats fed diets low in vitamins A and E with those of normal animals. He was unable to find consistent differences. It is to be noted, however, that the study was confined to nerve fibers and also that no details are given concerning the diets of the experimental animals.

More recently Wolbach and Bessey [20] and Mellanby [21] studied the rate of growth of nerve structures in relation to that of associated skeletal structures. They found that slow growth of skeletal elements accompanied by bone dysplasia causes excessive pressure which causes degeneration of neurons both directly and indirectly. Such an explanation appears to be untenable in the present instance; for, while the skeletal structures of A-deficient chicks grow more slowly than normal, their brains are also much smaller (fig. 1 vs. fig. 3), and there is no evidence of distortion of the brain.

COMMENT

The histologic abnormalities described in this paper show clearly that the condition of incoordination occurring in vitamin E-deficient chicks is associated with extensive destruction of the cerebellum supplemented by lesions of the brain stem. In vitamin A deficiency, however, incoordination is undoubtedly brought about by destruction of neurons of the brain stem and possibly also, to some extent, by visual difficulties occasioned by lesions occurring in the optic chiasma. It is also evident that the occurrence of pinpoint lesions is characteristic of vitamin A deficiency since no other type of lesion was found in these

14. Zimmerman, H. M.: J. Exper. Med. **57**:215, 1933.
15. Radhahrishna Rao, M. V.: Indian J. M. Research **24**:439, 1936.
16. Suzman, M. M.; Müller, G. L., and Ungley, E. C.: Am. J. Physiol. **101**:529, 1932.
17. Grinker, R. R., and Kandel, E.: Arch. Neurol. & Psychiat. **30**:1287, 1933.
18. Swank, R. L., and Davenport, H. A.: Stain Technol. **9**:11, 1934.
19. Duncan, D.: Arch. Neurol. & Psychiat. **25**:327, 1931.
20. Wolbach, S. B., and Bessey, O. A.: Arch. Path. **32**:689, 1941.
21. Mellanby, E.: J. Physiol. **101**:408, 1942.

brains except two in which a condition of vitamin E deficiency h. been deliberately superimposed. Even in these two only the small beginnings of lesions of E deficiency were found. In brains of E-deficie birds, on the other hand, degenerative changes of the cerebellum ar other parts of the brain were nearly always complicated by areas o. rarefaction similar to those seen in the brains of A-deficient birds. It must be concluded, therefore, that some degree of vitamin A deficiency had been established and that this was due to spontaneous changes in the E-deficient food, as maintained by Patrick and Morgan,[8] or is proof of a synergistic relation between vitamins A and E.

SUMMARY AND CONCLUSIONS

Young chicks reared on a ration deficient in vitamin A or a ration deficient in vitamin E show typical conditions of motor incoordination. These conditions occurring with the two vitamin deficiencies are difficult to distinguish on the basis of clinical symptoms, but both are associated with destruction of nerve cells in characteristic regions of the brain. In E-deficient chicks the lesions are found typically in the cerebellum, while in A-deficient chicks the lesions are mostly in the brain stem and the optic chiasma. Other regions of the brain may be affected in both deficiencies but the location of such lesions is variable.

Microscopic study has shown that the brain lesions are clearly diagnostic of vitamin A or vitamin E deficiency and may be distinguished on the following basis.

Vitamin E deficiency:

Gross lesions: General disintegration of the cerebellum, accompanied by greenish yellow discoloration, hemorrhage and edema. Rarely lesions occur in the cerebrum.

Microscopic lesions: Degeneration of neurons in the affected areas, characterized by pyknosis and shrinkage of cell bodies; degeneration of nerve fibers, accompanied by formation of blister-like vesicles on the fibers.

Vitamin A deficiency:

Gross lesions: None.

Microscopic lesions: Pinpoint areas of degeneration in the brain stem, the base of the cerebellum, the optic chiasma and (rarely) in the cerebrum. Degenerating cells lose their chromaticity but do not undergo shrinkage. Nissl substance breaks up into fine granules and filaments before undergoing complete dissolution.

Although the E-deficient birds were receiving vitamin A supplements at levels which should have given complete protection, the fact that lesions characteristic of A deficiency regularly occurred in their brains suggests that an intimate relationship exists between the two vitamins. It is clear that either the relation is a synergistic one or vitamin E has a protective role in relation to vitamin A.

ADAMANTINOMA IN THE TIBIA

BÉLA HALPERT, M.D.
OKLAHOMA CITY
AND
H. PHILLIP DOHN, M.D.
MILWAUKEE

EPITHELIAL neoplasms mimicking the structure of the budding enamel body and called adamantinoma or ameloblastoma usually occur in the jaw.[1] Craniopharyngioma, a neoplasm with similar or identical structure, is occasionally observed at the base of the skull.[2] A case of adamantinoma of the ulna [3] and several of adamantinoma of the tibia have been described. Recently Hebbel [4] tabulated 15 cases of adamantinoma of the tibia, to which 2 cases reported by Dockerty and Meyerding [5] and 1 by Cagnoli [6] have been added since. Because of the problems which these growths present as to cellular origin, diagnosis and treatment, the following case is reported in some detail.

A 24 year old white woman was admitted to the University of Oklahoma Hospitals, Oklahoma City, Nov. 14, 1945, with the complaint of a painful swelling of the middle and lower portions of the right leg. She stated that in August 1943 she had fallen and struck her leg against a step. The leg was painful for several weeks, but she was able to carry on her usual activities. The painful area, over the crest of the tibia, about 4 inches (10 cm.) proximal to the ankle joint, gradually decreased. In February 1944 the pain returned, necessitating the use of a crutch in walking. She discarded the crutch early in April. About three weeks later she slipped on a marble floor and "broke her leg." The fracture was reduced without anesthesia and a boot cast applied. The cast was removed in six weeks. When bearing of weight was resumed two weeks later, painful swelling of the leg followed. The swelling increased, and the pain persisted. In October 1945 a roentgenographic examination disclosed a "bone tumor," and amputation was advised.

On admission she appeared well developed, with evidence of some recent loss of weight. Systemic examination gave essentially negative results. There was a

From the Department of Pathology and the Department of Orthopedic Surgery, University of Oklahoma School of Medicine.

1. Robinson, H. B. G.: Arch. Path. **23**:831, 1937. Chont, L. K.: Am. J. Roentgenol. **50**:480, 1943.

2. Globus, J. H., and Gang, K. M.: J. Mt. Sinai Hosp. **12**:220, 1945.

3. Anderson, C. E., and Saunders, J. B. de C. M.: Surg., Gynec. & Obst. **75**:351, 1942.

4. Hebbel, R.: Surgery **7**:860, 1940.

5. Dockerty, M. B., and Meyerding, H. W.: J. A. M. A. **119**:932, 1942.

6. Cagnoli, H.: Bol. Soc. cir. d. Uruguay **14**:305, 1943; Arch. urug. de med., cir. y especialid. **24**:237, 1944.

fusiform enlargement of the middle of the right leg, extending to within 3 cm. of the ankle joint. The mass was nonfluctuant and had a bony consistency. The overlying skin was tense, shiny and nonadherent, and there was no increase in local heat. The mass was apparently continuous with the tibia. There was moderate swelling of soft tissues over the fibula and the adjacent regions, with no apparent involvement of the fibula. The pulse in the dorsalis pedis artery was feeble, and the capillary circulation in the beds of the toe nails was poor.

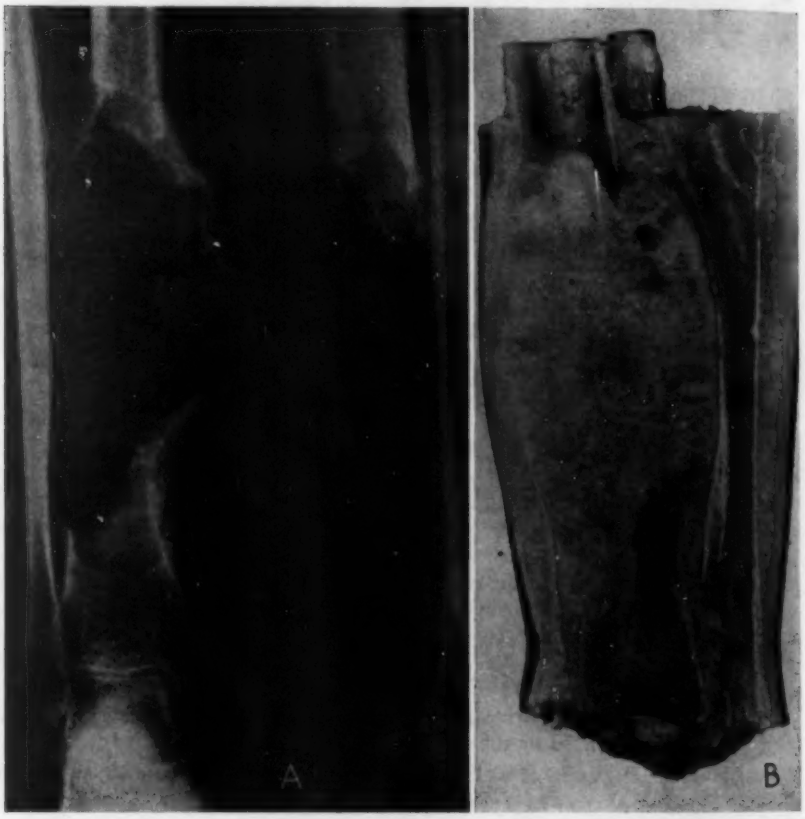

Fig. 1.—*A*, roentgenogram of the tibia, showing an osteolytic lesion destroying the shaft for a length of about 12 cm. *B*, cut surface of the leg, exposing the area of neoplastic involvement.

Roentgenograms disclosed an osteolytic mass which had destroyed approximately 12 cm. of the distal part of the shaft of the tibia (fig. 1 *A*). There was expansion of the bony cortex, with only a thin shell remaining. The fibula and the ankle joint appeared intact. Roentgenograms of the chest disclosed no involvement of osseous or soft tissues.

The urine was neutral, cloudy, with a specific gravity of 1.030; it revealed no albumin and no sugar. The red blood cell count was 3,980,000; the hemoglobin content was 12 Gm.; the white blood cell count was 14,800, with polymorphonuclears

79, eosinophils 2, monocytes 1 and lymphocytes 18 per cent. The Mazzini and Kolmer-Wassermann tests of the blood were both strongly positive on two occasions.

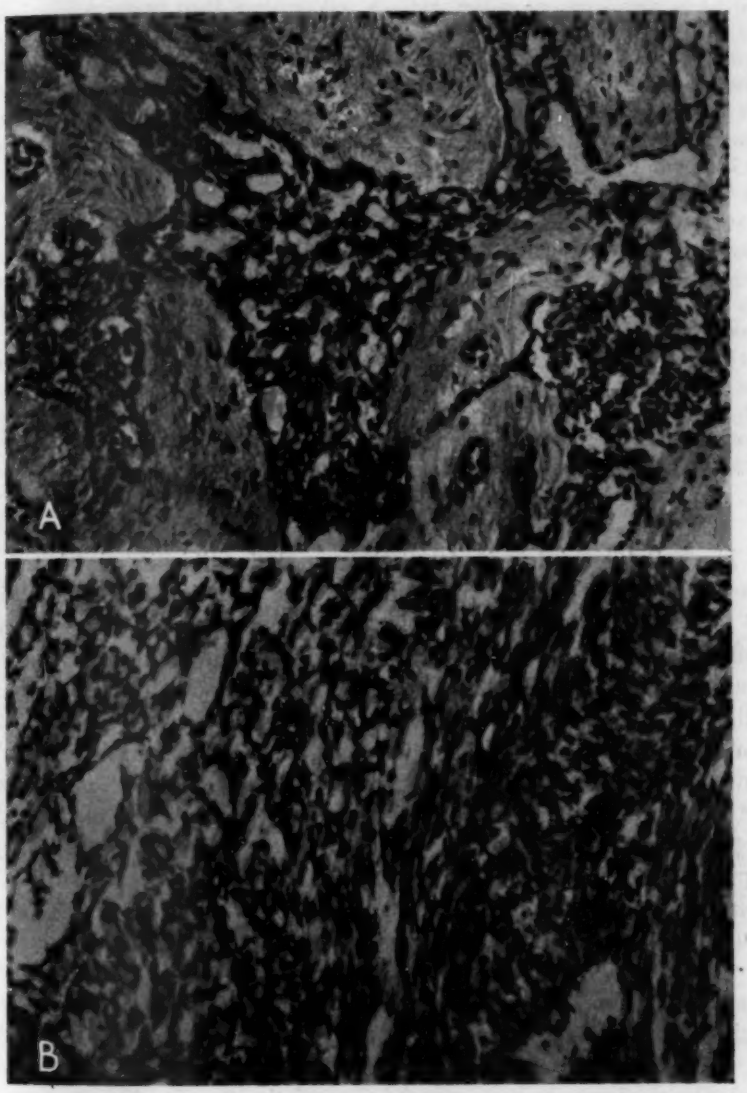

Fig. 2.—Microscopic appearance of the growth. *A*, shows the palisade arrangement of the peripheral cells. In the center of each cell nest there is a stream or whorl-like arrangement of the cells and a lacelike pattern with small and large spaces between cells. × 225.

The clinical impression was: osteolytic bone tumor. On Nov. 17, 1945 the leg was amputated at the junction of the middle and distal thirds of the right thigh, with primary closure of the stump. The postoperative course was uneventful, and

the patient was discharged nineteen days later. When she returned on April 4, 1946, the stump was well healed and she was in good health.

On the extremity, amputated 12 cm. proximal to the knee joint, there were no erosions or discolorations of the skin. A subcutaneous hard fusiform prominence, 8 by 6 by 2 cm., apparently continuous with the tibia was felt commencing about 9 cm. above the level of the inner malleolus. On the cut surfaces (fig. 1 *B*) gray-white neoplastic tissue filled the marrow cavity of the tibia and replaced the compact layer, extending through it into the soft tissues. A convex line of demarcation separated the growth from the soft tissues laterally and a similar wavy convex line bordered it proximally and distally. The growth involved an area about 15 cm. long and 8 cm. wide.

Microscopic preparations stained with hematoxylin and eosin, representing many parts of the growth, disclosed sheets and nests of epithelial cells in a loose fibrous connective tissue stroma. In places the cells formed streams or whorls, with a palisade arrangement of the peripheral cells (fig. 2 *A*). They had deeply stained large round or oval vesicular nuclei with a hardly discernible cytoplasm, which faded into a delicate fibrillar substance. A number of cells were seen in a state of division. In some of the cell nests the cells were in a lacelike pattern, with small or large spaces between them (fig. 2 *B*). Dense hyalinizing fibrous connective tissue bordered the growth and was invaded by cell nests. Apparently compressed striated muscle bundles were seen outside the capsule of the growth.

COMMENT

The cellular origin of adamantinoma of the tibia has intrigued all observers since Fischer[7] reported the first case in 1913. Epithelial cell rests are frequently found about the jaws and about the base of the skull in the region of the sella turcica. Adamantinoma most frequently occurs at these sites. Epithelial cell rests have not actually been observed in the tibia, yet the microscopic structure of these growths arising in the tibia is similar to, if not identical with, that of adamantinoma or of craniopharyngioma. The skin over the growth remains uninvolved. The growth extends from inside the bone outward. These observations suggest that adamantinoma of the tibia, like that occurring elsewhere, is probably derived from an embryonal rest of the ectoderm. The environment of adamantinoma is similar at all sites where the tumor occurs: that is to say, the growth is in close proximity to periosteum or to osseous tissue. The simple range of variation of the cellular pattern of adamantinoma suggests that the tumor is of more recent genetic origin than the anlage tumors of the salivary glands. In the latter the stroma is a part of the neoplasm whereas in adamantinoma and craniopharyngioma it is a contribution of the surrounding connective tissue.[8]

Adamantinoma is essentially a benign neoplasm with locally invasive properties. It grows slowly and usually shows no areas of necrosis or

7. Fischer, B.: Ztschr. f. Path. **12**:422, 1913.
8. Halpert, B.: Cancer Research **6**:504, 1946.

hemorrhage. Cells in a state of division, however, are frequent in many parts of the growth. No distant metastases have been observed in cases of adamantinoma of the tibia,[9] although a case of Dr. Henry F. Hunt, of the George F. Geisinger Memorial Hospital, Danville, Pa., as yet not reported, appears to be the first instance in which the tumor has metastasized. The growth may be recognized by its characteristic clinical behavior and roentgenographic appearance. Early amputation is the treatment of choice.

SUMMARY

An epithelial anlage tumor—adamantinoma—of the tibia of a 24 year old white woman is reported. This is the nineteenth recorded instance of such a growth of the tibia.

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PARACOLON BACILLUS ENDOCARDITIS OF THE PULMONIC VALVE SECONDARY TO INFECTED POLYCYSTIC KIDNEYS

THEODORE ROBERTSON, M.D.
NEW YORK

THIS is the report of a case of endocarditis of the pulmonic valve due to a paracolon bacillus and occurring secondarily to abscesses of polycystic kidneys. No report of a case of paracolon bacillus endocarditis has been found in the literature.

Bacterial endocarditis of the pulmonic valve is relatively rare, and solitary infection of this valve extremely so.[1] In most of the cases the lesion has occurred on a valve with a congenital or an acquired malformation.[2] Few cases of solitary bacterial endocarditis occurring on a previously undamaged pulmonic valve have been reported.[3] In a survey of 7,000 autopsies performed at the New York Hospital and the Cornell University Medical College since 1917 no other cases of bacterial endocarditis of a previously undamaged pulmonic valve have been found.

Organisms of the Bacterieae group seldom are primary in bacterial endocarditis. Schilling[4] listed reports of endocarditis due to Escherichia coli,[5] Escherichia acidi lactici,[6] Eberthella typhosa (or typhi) and Aerobacter aerogenes. Wells observed a case in which "Bacillus paratyphosus B" (Salmonella paratyphi B) was the causative organism.[7] Such endocarditis is often secondary to infections of the abdominal or pelvic cavities.

The paracolon bacillus was first recognized as a distinct organism by Mair in 1906.[8] He found it associated with acute infections of the urinary tract. However, general acceptance of it as a separate organ-

From the Department of Pathology, Cornell University Medical College.

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4. Schilling, M. S.: Ann. Int. Med. **13**:476, 1939.

5. Harries, G. E., and Burtenshaw, J. M. L.: Lancet **2**:803, 1937.

6. Dickar, L.: Arch. Int. Med. **44**:788, 1932.

7. Wells, H. G.: Arch. Path. **23**:270, 1937.

8. Mair, W.: Brit. M. J. **1**:438, 1906.

ism has been recent. It is a gram-negative, seldom motile, rod-shaped organism with colonies resembling those of Bacillus coli in form but dirty white with a suggestion of pink in the center on McConkey's agar medium. It is regarded as an intermediate between B. coli and the paratyphoid group and differs culturally from B. coli in that it ferments lactose slowly, poorly or not at all. It differs biochemically from the paratyphoid group in that it gives a positive indole reaction, and it also does not agglutinate with paratyphoid agglutinating serums. A similar, hemolytic, organism was isolated by Dudgeon from acute infective processes involving the gastrointestinal and urinary tracts. He mentioned two deaths, one of an infant with multiple septic foci in the renal cortices and the other of a patient with "'acute pyelocystitis." In both the paracolon bacillus was apparently the infective agent.[9]

REPORT OF A CASE

A 40 year old white man, a waiter, stated that a sharp pain developed in the left lower quadrant of the abdomen rather suddenly in January 1946, with hematuria, urgency and frequency. A diagnosis of polycystic kidneys was made, and nephrocystotomy was performed on the left side at that time. The wound became secondarily infected and drained for some time, then gradually healed.

In May 1946, while in a nursing home, he had a sudden onset of severe cramping spasmodic periumbilical pain which lasted a few hours and recurred the next day with chills, fever and frank hematuria. After four days he was admitted to the New York Hospital. His pulse rate was 90, respiratory rate 25, and blood pressure 150 systolic and 90 diastolic. One observer noted slight enlargement of the heart and a basal systolic murmur. Studies indicated an obstruction of the common bile duct, and at operation, May 23, a chronically inflamed gallbladder and a stone of the common bile duct were removed. Two blood cultures made before operation were sterile. A perinephric abscess was first noted on the sixth day after operation, and two days later it was incised and drained. Culture of material taken from this abscess on two occasions revealed only paracolon bacilli. A blood culture made on the day of incision of the abscess showed only paracolon bacilli, as did another made seven days later. For two weeks after operation the patient had a rapid pulse (rate, 95 to 120) but no fever. Ten days postoperatively a fistula developed from his perinephric abscess, and a biliary fistula as well, with profuse drainage. From his fourteenth postoperative day on he had daily elevations of temperature reaching approximately 38 C. (100.4 F.). On his eighteenth postoperative day a fecal fistula appeared in the lower part of the abdominal wound.

He had received 50,000 units of penicillin intramuscularly every three hours during the first nineteen days after the operation; thereafter he received 4 Gm. of sulfadiazine daily by mouth. Uremia, which was moderate on admission, gradually increased, and edema appeared. His anemia increased despite many transfusions of whole blood. Acidosis developed and rapidly became more severe. His temperature never rose above 38.4 C. (101.1 F.) despite his overwhelming infection. At the time of his death on the fifty-fourth day following cholescystectomy the blood urea nitrogen was 136 mg. per hundred cubic centimeters. No changes of cardiac

9. Dudgeon, L. S., and Pulvertaft, R. J. V.: J. Hyg. **26**:285, 1927. Dudgeon, L. S.; Wordley, E., and Bawtree, I.: ibid. **21**:168, 1922.

status were noted during his illness, and no petechiae were seen in the skin or the mucous membranes.

Autopsy (two hours after death).—The body was cachectic, weighing only 45 Kg. There was a slight yellow tint to the skin and the scleras. The biliary fistula drained a main radical of the hepatic duct. The fecal fistula extended in over the upper pole of the left kidney, between it and the spleen, and was apparently continuous with the peritoneal cavity at this point; many adhesions were noted here. The kidneys were huge and occupied much of the abdomen. The right kidney weighed 1,380 and the left 1,020 Gm. The capsules were from 3 to 5 cm. in diameter. Some of the cysts contained blood and some thick, cloudy fluid but most contained thick, foul-smelling purulent material. Little functioning renal tissue remained. The pelves were stretched, but the ureteropelvic junctions and the ureters were not remarkable.

The heart weighed 400 Gm. and was globular in shape with predominance of the left ventricle. The walls of the left and right ventricles were 24 and 7 mm. thick,

Fig. 1.—Endocarditis of the pulmonic valve and adjacent endocardium due to the paracolon bacillus. Note the large vegetation on the left anterior cusp. Approximately the actual size is shown.

respectively. The endocardium was thin and delicate except in the region of the pulmonic valve (fig. 1). Here there were numerous firm, flattened, yellow vegetations in the endocardium of the pulmonary conus and the pulmonary artery. The cusps of the pulmonic valve were almost entirely destroyed by similar vegetations. One huge vegetation (4 by 3 by 0.5 cm.) was attached to the left anterior cusp and flapped in the conus like an accessory valve. It was composed of firm, yellow granulation tissue and was not ulcerated. Many of the smaller vegetations were ulcerated. The bases of the three cusps were delicate, and there was no fusion of the commissures. The valve ring was 7 cm. in circumference. The other valves were thin and delicate, and their chordae tendineae were thin and not fused. The coronary arteries had little sclerosis.

The lungs weighed 600 Gm., and the vessels were prominent. The spleen weighed 550 Gm. and was dark red and firm. The liver weighed 2,450 Gm. and was red-brown and firm. The hepatic and common bile ducts were not obstructed. The other viscera were not remarkable. The brain was not examined.

Bacteriologic Examination.—Postmortem cultures were made of the heart's blood, the right lower lobe of the lung, and pus from the cysts of the right and left kidneys. A piece of the large vegetation of the pulmonic valve was put in 70 per cent alcohol for about five minutes and then placed in a sterile Petri dish. Later it was crushed and cultured. A gram-negative rod-shaped bacillus that was culturally and biochemically a paracolon bacillus was grown from each of these sites. From the heart, the lung and the vegetation the paracolon bacillus was the sole organism grown, but from the kidneys E. coli, Clostridium welchii and a nonhemolytic streptococcus, gamma type, were also grown. The requirements met in its isolation were that it gave a positive indole reaction; it produced no fermentation in lactose during ten days' observation; it produced acid and gas in dextrose, maltose, mannite, xylose, arabinose, salicin and sorbitol. It gave no reaction in saccharose, inosite, urea, lead acetate and gelatin. It produced acid slowly in litmus milk. No attempt was made to classify the paracolon bacillus into a subgroup.

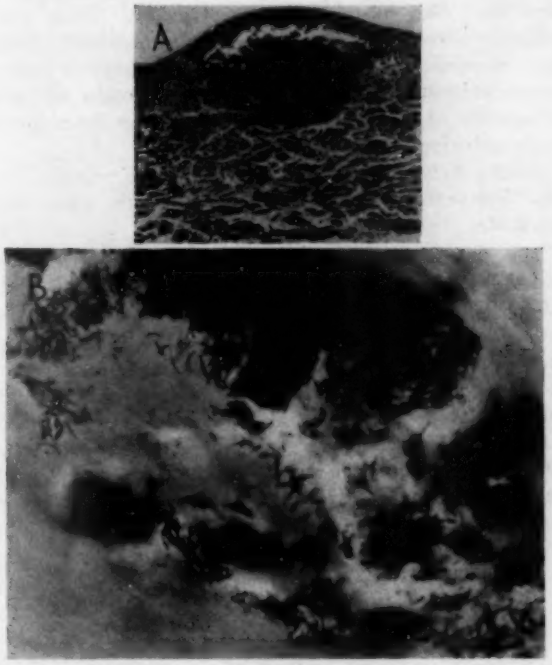

Fig. 2.—*A*, acute lesion of the pulmonary conus, magnified approximately 90 times; hematoxylin and eosin stain. Lesions larger than this were ulcerated. Here the myocardium is infiltrated to some extent by inflammatory cells.

B, paracolon bacilli in the large vegetation, magnified approximately 700 times; Giemsa stain.

Microscopic Examination.—Sections of the pulmonic valve and the pulmonary conus and artery stained with hematoxylin and eosin revealed beneath the endocardium a fibroblastic proliferation which in places showed mucoid degeneration. The vegetations seen in the gross were composed of focal thickenings due to the fibroblastic proliferation and cellular infiltration accompanying a fibrinopurulent process that was causing destruction of the endocardium (fig. 2 *A*). The infiltrating

cells were mostly polymorphonuclear leukocytes. Small rod-shaped bacteria were present in large numbers in the vegetations examined. With a modified Brown's stain for bacteria (Gram) these stained a brilliant red. No other bacteria were seen in an extensive examination (fig. 2*B*). The myocardium of the pulmonary conus was infiltrated by leukocytes in areas directly beneath the vegetations. However, the media of the pulmonary artery was infiltrated by both leukocytes and fibroblasts for a considerable area.

Sections of the lungs revealed marked congestion of the capillaries and some atelectasis, as well as thickening of the walls of the arterioles and small arteries.

Hematoxylin and eosin–stained sections of both kidneys revealed the capsules moderately thickened and almost the entire parenchyma replaced by cysts and fibrous tissue. The cysts contained blood, precipitated protein, or purulent material consisting of hemolyzed red blood cells and many polymorphonuclear leukocytes. The cysts were lined with cuboidal or flat epithelium. There were many areas both of coagulation necrosis and of mucinous degeneration. Hemosiderin-laden phagocytes were numerous in the fibrosed areas. A small area of comparatively uninvolved cortex was noted, but even here one saw atrophy and necrosis of some tubules and congestion of the glomeruli. In other areas the cortex was markedly compressed by neighboring cysts.

Similarly stained sections of the parathyroid glands revealed small, relatively inactive glands. The pancreas was fibrotic. The liver, the spleen and other organs were not remarkable.

Anatomic Diagnosis.—Infected polycystic kidneys (culture reveals paracolon bacilli, E. coli and Cl. welchii); fistula from the upper pole of the left kidney to the skin of the flank; uremia (blood urea nitrogen, 66 mg. per hundred cubic centimeters); slight hypertrophy of the heart; bacterial endocarditis of the pulmonic valve (culture reveals paracolon bacilli). (The leaflets of the pulmonic valve are almost completely destroyed and are covered with vegetations, many of them small and one large. There are also many small vegetations in the endocardium of the neighboring pulmonary conus and artery. Small rod-shaped gram-negative bacteria are present in large numbers in these lesions.) There were chronic cholecystitis, cholelithiasis and choledocholithiasis, with operative removal of gallbladder and stones; biliary fistula to the right upper quadrant of the abdomen; fecal fistula from the cecum to the right lower quadrant of the abdomen; cachexia (height, 174 cm.; weight 45 Kg.); marked arteriosclerosis of the small arteries and arterioles of the lungs and slight sclerosis of the aorta and coronary arteries.

COMMENT

That bacterial endocarditis may be a consequence of septicemia which, in turn, is a consequence of an acute or chronic infectious process occurring elsewhere in the body is generally accepted. It is also known that the causative agent of bacterial endocarditis may be any one of a large number of bacteria. The mechanism of production of such endocarditis is still not agreed on, and this case gives support to no particular hypothesis. It is generally considered that a previously damaged valve is more apt to be invaded by bacteria than an undamaged one. The pulmonic valve is the least frequently affected by bacterial endocarditis of all the cardiac valves. In only 1 per cent or less of several recorded series of cases of this disease has the pulmonic valve alone been the seat of a bacterial lesion as it was in this case.[1]

Careful gross and microscopic examination of the heart in this case failed to reveal any evidence of valvular lesions preceding the current one. The leaflets of the aortic, mitral and tricuspid valves were thin and delicate. There was no vascularization of the cusps, no fusion at the commissures, no thickening of the chordae tendineae. The bases of the cusps of the pulmonic valve were delicate, and there was no fusion at the commissures. The usual three cusps were present. Microscopically, there was no vascularization of the remnants of the cusps and no fibroblastic proliferation away from the acutely infected areas. Therefore, it is probable that there was no preexisting damage of this valve.

As no one has previously identified the paracolon bacillus with lesions other than those of the kidney, it is necessary to rule out the possibility that there was in the lesion of the valve a coexisting or a preexisting organism. Cultures of the perinephric abscess and of the blood made during life produced only pure growths of the paracolon bacillus. Cultures of pus taken from cysts of the right and the left kidney showed growths of the paracolon bacillus, E. coli, Cl. welchii and a nonhemolytic streptococcus, gamma type. Careful search of Giemsa-stained sections of the vegetations revealed only small rod-shaped bacteria. Gram stains showed only gram-negative organisms in the vegetations. Some investigators have suggested that the paracolon bacillus results when a strain of E. coli is modified in the body of the host and, to support this contention, have presented evidence of changing and variable cultures of material taken from the same subject over a period of time.[10] However, it seems reasonable to assume that in this case the endocarditis was initiated and maintained by a paracolon bacillus infection alone. There is no evidence here to support the theory that an organism has been modified within the host.

Chemotherapy was ineffective in controlling this infection. The lesions in the pulmonary conus and artery were acute, involving the myocardium and the media. The extreme debility of this patient, due to chronic renal insufficiency and acute obstruction of the biliary tract, may have lowered his resistance to what is ordinarily a weakly pathogenic organism.

SUMMARY

A case of massive vegetative endocarditis of the pulmonic valve due to the paracolon bacillus is reported. The other cardiac valves were normal. The valvular lesion and the septicemia were secondary to infected polycystic kidneys. No report of a similar case has been found in the literature.

10. Schaub, I. G.: J. Lab. & Clin. Med. **31**:958, 1946.

HODGKIN'S SARCOMA OF UTERUS

HANS N. NAUMANN, M.D., TAUNTON, MASS.

THE STUDY of Hodgkin's disease has received a new impetus from the recent comprehensive investigations of Jackson and Parker,[1] who introduced the concept of three clearly defined clinical and pathologic entities of the disease, namely, Hodgkin's paragranuloma, granuloma and sarcoma. The case to be reported falls into the third group of this classification and is noteworthy for the unusual involvement of the uterus and the extremely advanced age of the patient.

REPORT OF A CASE

M. A. S., an 81 year old white woman, was admitted to the Taunton State Hospital and her condition diagnosed as senile psychosis. The father and one brother had died of cancer. The patient had never been seriously ill. She had been married for sixty-two years and had three children. The mental illness began two years prior to admission with irritability, silly actions, such as cutting up clothes and giving away household objects, and ideas that she was persecuted by her daughter, with whom she was living. On admission she was found to be a senile patient of 105 pounds (47.5 Kg.) and of 59 inches (148 cm.) in height. The blood pressure was 165 systolic and 70 diastolic. Physical, neurologic and vaginal examinations gave negative results. The hemoglobin content was 10.6 Gm.; the red blood cell count, 3,859,000; the white blood cell count, 6,500, with a differential count of 70 per cent polymorphonuclear cells, 8 per cent stab cells, 1 per cent eosinophil cells and 21 per cent lymphocytes. The blood was of type A. The Kahn and Hinton tests were negative. The urine was acid, with a specific gravity of 1.024, and there were no significant chemical or microscopic changes. A stool was negative for enteric pathogens. After a nine months' observation period, during which she had been tidy and cooperative, the patient was released to go home for a trial visit. However, a few months later she became unmanageable again and was returned to the hospital. The subsequent course was generally unchanged until her death, which took place with the signs of heart failure about two years after her admission.

Autopsy (thirteen hours after death).—The body was that of an 83 year old white emaciated woman measuring 59 inches in length and weighing about 90 pounds (about 41 Kg.). There was extreme wasting of adipose and muscular tissues. The sacral region and buttocks showed a large decubital ulcer. The finger nails and the lips were cyanotic. The right pupil was larger than the left.

On opening the peritoneal cavity a markedly enlarged uterus was found projecting domelike from the lesser pelvis above the symphysis. It measured 7 by 10 by 15 cm. and corresponded in size to that of about four months' gestation. The upper surface was covered with smooth serosa; the lower portion of the

From the Department of Pathology, Taunton State Hospital, Taunton, Mass.

1. Jackson, H., Jr., and Parker, F., Jr.: New England J. Med. **230**:1, 1944; **231**:35 and 639, 1944.

corpus was continuous with fused infiltrated lymph nodes lining the wall of the lesser pelvis and extending upward around the aorta. These masses of tumor tissue surrounded all structures, especially vessels of the lesser pelvis, without causing obstruction except of the right ureter slightly above its juncture with the bladder. The cervix was not involved and was normal except for old lacerations of the external orifice. The uterine cavity was compressed and displaced to the anterior and left side. The endometrium was thin and tan colored, with some small hemorrhages in the fundus. The myometrium of the posterior wall was completely replaced by white tumor tissue with some necrotic and hemorrhagic areas in the center. There was also diffuse infiltration of the anterior and the lateral wall of the myometrium. The adnexa attached to the lateral aspects of the upper, serosa-covered portion of the uterus were atrophic and free of tumor tissue. The vulva and vagina were atrophic.

The lymphoid system showed, in addition to the infiltrated pelvic and aortic nodes, some enlarged mesenteric nodes. On section these nodes were white and firm, with occasional areas of necrosis. The spleen weighed 90 Gm. and showed a smooth capsule and soft hemorrhagic pulp.

The heart weighed 200 Gm. and was flabby. The myocardium showed brown atrophy. The aortic valves and the coronary arteries were sclerosed and partly calcified. The aorta and the major arteries showed a moderate degree of arteriosclerosis. The lungs were congested, the lower lobes edematous and the upper lobes moderately emphysematous, with old apical scars.

The intestines were not remarkable except for the rectum, which was surrounded by tumor tissue at the level of the pelvic floor. The pancreas weighed 90 Gm.; the liver, 990 Gm. Both organs, as well as the gallbladder and the bile ducts, were without gross changes.

The kidneys showed marked contraction, the left weighing 50 Gm. and the right 30 Gm. The capsules stripped with moderate difficulty leaving finely granular surfaces. On section the right kidney showed evidence of hydronephrosis, marked dilatation of the pelvis and calices and atrophy of the parenchyma, which in places measured as little as 4 mm. in thickness. The left kidney showed a normal pelvis but narrowing of the cortex and medulla. The right ureter was compressed by tumor tissue near the bladder and above this point was dilated up to 8 mm. in diameter. The bladder was surrounded at the fundus by tumor tissue, which, however, did not infiltrate its wall.

The adrenal glands weighed 5 Gm. and showed some autolytic changes. The pituitary gland was of normal size and shape. There was marked osteoporosis of ribs and skull. The central nervous system showed adherence of the dura to the calvarium, fibrosis of the arachnoid and marked arteriosclerosis. The brain after fixation in solution of formaldehyde weighed 1,360 Gm. and showed moderate cortical atrophy of the frontal and parietal lobes. Coronal sections revealed a cavity measuring 5 mm. in diameter in the right lentiform nucleus and a small area of softening in the white matter of the right frontal lobe.

Microscopic Examination.—Microscopic sections of the uterine fundus (fig.) showed mainly tumor tissue, which in some areas was seen infiltrating between smooth muscle fibers. The tumor cells, which were arranged in sheets with partly scanty, partly dense stroma, were markedly pleomorphic, with hyperchromatic nuclei and cytoplasm varying in staining reaction from basophilic to acidophilic. The most conspicuous cells were giant cells (fig. 2) measuring from 15 to 35 microns; many were multinucleated, and many showed large round, lobulated or indented nuclei. The latter consisted of mostly dark staining, irregular chrom-

atin; some contained prominent nucleoli. Some cells showed mitotic figures. The cytoplasm of these giant cells varied also in staining reaction from basophilic to slate colored to acidophilic. Blood vessels were abundant and consisted of single layers of endothelial cells. There were large areas of hemorrhage and necrosis. One

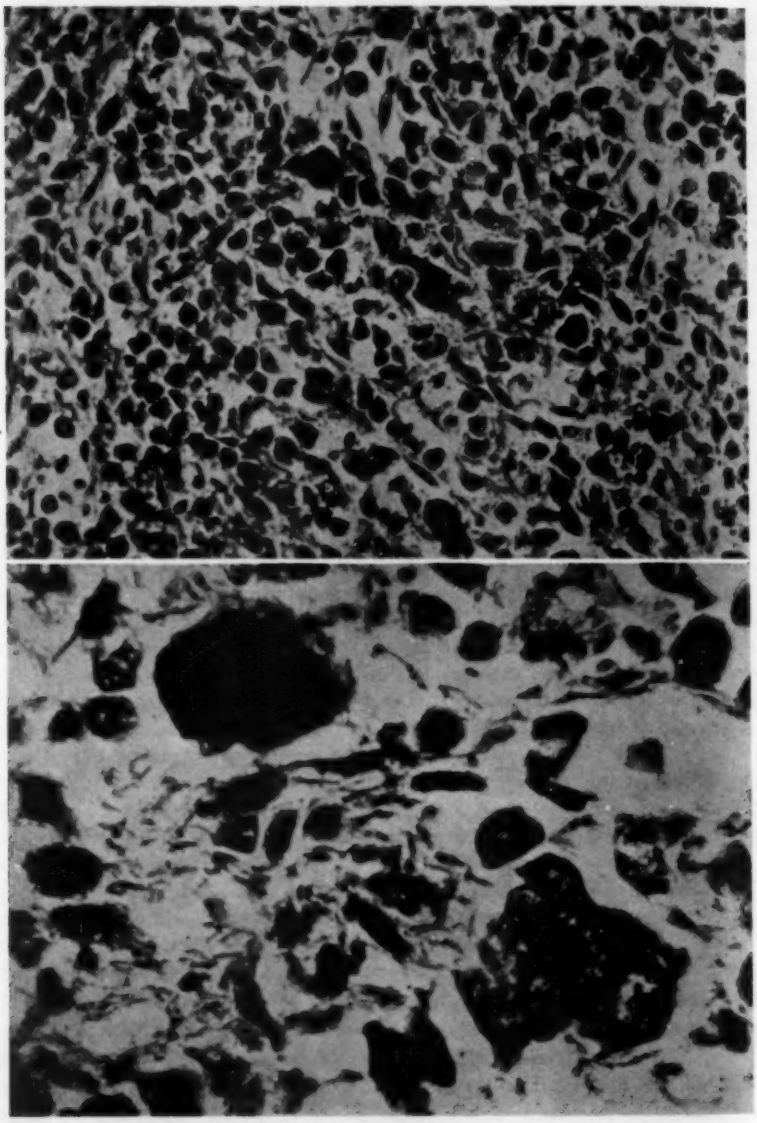

Fig. 1.—Typical field of uterine tumor showing pleomorphism and Reed-Sternberg cells. Hematoxylin and phloxine; × 250.

Fig. 2.—Two large cells of Reed-Sternberg type. Hematoxylin and phloxine; × 900.

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TABLE 1.—*Summary of Cases Reported Since 1912*

Author	Age	Organs Involved	Uterus		Other Data
			Macroscopic	Microscopic	
Uddströmer [4] (1933)	39	Lymphatics, spleen, liver, mesentery, uterus	15 cm. greatest dimension; diffuse infiltration: endometrium necrotic	Hodgkin's sarcoma, infiltrating between muscles; vessels thrombosed	Duration 1½ years
Lewinski [3] (1930)	43	Lymphatics, spleen, liver, bone marrow, lungs, kidneys, bladder, urethra, skin, peritoneum, vagina, uterus, tubes	Nodes in serosa; infiltration and necrosis of cervix resembling cancer	Hodgkin's granuloma	Cachexia; white blood cell count from 2,020 to 28,000
Scenes [2] (1929)	35	Lymphatics, tonsils, pharynx, intestine, spleen, pleura, bladder, ovaries, tubes, uterus	9 by 5 by 1.6 cm.; intramural white tumor with indistinct demarcation; white nodes in serosa	Hodgkin's granuloma	Anemia; white blood cell count 16,800; dilated heart
Walthard, K. M.: Ztschr. f. d. ges. Neurol. u. Psychiat. **97**: 1, 1925	35	Spinal canal (extradural), uterus	White nodule, 1.1 by 1.1 by 0.7 cm.; border not sharp	.....	Incomplete autopsy
Schlagenhaufer, F.: Arch. f. Gynäk. **95**: 7, 1912	68	Lymphatics, spleen, pancreas, mesosalpinx, mesovary, plexus uterovaginalis	Massive growth in plexus uterovaginalis	.....	Incomplete description
Jessup, W. E. D.: Proc. New York Path. Soc. **12**: 3, 1912	43	Lymphatics, spleen, skin, tubes, one ovary; simultaneous tuberculosis	12 by 9 by 6 cm.; diffuse infiltration, fibrosis	Hodgkin's granuloma	
Present author (1946).....	83	Lymphatics, uterus	15 cm. greatest dimension; diffuse infiltration, neurosis	Hodgkin's sarcoma	Duration less than 2 years; no clinical signs

region showed endometrial glands. Silver impregnations revealed a rather dense network of reticulum fibers enmeshing tumor cells.

Sections of the cervix showed diffuse infiltration with tumor tissue identical in type with that described in the fundus uteri. There were areas of well preserved myometrium.

The aorta showed marked intimal deposits of lipids and calcium. Adjacent to the adventitia there were remnants of lympoid tissue infiltrated diffusely by tumor cells identical with those described.

COMMENT

The histologic features of marked pleomorphism, of giant cells resembling those of Reed and Sternberg and of reticulum can be considered characteristic of Hodgkin's sarcoma, which in this case was restricted to the abdominal lymphatic system and uterus. The preferential involvement of the lymphoid system in Hodgkin's disease makes it plausible to assume that the process originated in the pelvic nodes and from there invaded the uterus, the posterior wall of which was the main site of infiltration.

TABLE 2.—*Hodgkin's Sarcoma in Old Persons*

Author	Cases	Age
Krueger and Meyer [6]	60	Oldest 74 ♀ 84 ♂
Uddströmer, M.: Acta tubere. Scandinav., 1934, supp. 1, p. 1	536	5 months to 77
Williams, J. E., and Oliver, T. M.: Texas State J. Med. 32 : 486, 1936	11	12 ♀ Negro to 77 ♂ white
Goldman, L. B.: J. A. M. A. 114 : 1611, 1940	212	6 to 76
Bruger, R. E., and Lehman, E. P.: Arch. Surg. 43 : 839, 1941	54	4 to 79
Baker, C., and Mann, W. N.: Guy's Hosp. Rep. 89 : 83, 1939	65	7 to 67
Haden, R. L., and Burns, J. T.: Cleveland Clin. Quart. 9 : 144, 1942	47	3½ to 67
Stewart, F. W., and Doan, C. H.: Ann. Surg. 93 : 141, 1931	24	16 (1 ♂ 1 ♀) to 57
Jackson and Parker [1]		
Paragranuloma	38	8th decade 1 patient
Granuloma	237	9th decade 1 patient
Sarcoma	51	8th decade 7 patients
Saller, S.: Am. J. Clin. Path. 6 : 241, 1936	74	8th decade 2 ♂ 1 ♀
Mills, E. S., and Pritchard, J. E.: Canad. M. A. J. 33 : 50, 1935	20	8th decade 1 patient

An opinion on the incidence of uterine involvement in Hodgkin's disease can be gained from Jackson and Parker's large series comprising a total of 326 cases of all types. On detailed analysis these authors reported that of 59 cases of Hodgkin's granuloma with autopsy the uterus was involved in 2 and that among 27 cases of Hodgkin's sarcoma there was 1 with uterine involvement—an incidence of 3.4 per cent and 3.7 per cent, respectively. On surveying the more accessible literature of the past forty years, reports on 6 more cases were found as compiled in table 1. In all these cases the involvement of the female sex organs represents only one localization of a widespread process which may invade all portions of the genital tract as in Scenes'[2]

2. Scenes, A.: Ztschr. f. Geburtsh. u. Gynäk. 96:121, 1929.

case or parts of it as in Lewinski's [3] case or the uterus selectively as in Uddströmer's [4] and the present case. On the other hand, the ovaries may be affected selectively and the uterus spared as in 1 of the 2 cases recently reported by Heller and Palin.[5] These authors classified their case as an instance of Hodgkin's sarcoma and demonstrated tumor cells within veins suggesting a spread through the blood circulation. It is interesting to note the close resemblance between Uddströmer's and the present case in the histologic appearance of the tumors, as well as in the diffuse infiltration of the myometrium resulting in uterine enlargement of identical proportion, and in duration of the disease.

Another unusual aspect of the present case is the advanced age of the patient, who died at 83 years. Data compiled from the literature in table 2 show only 1 patient older than the present one, a man aged 84 years, whose case was reported by Krueger and Meyer,[6] and only 1 among Jackson and Parker's 237 patients with Hodgkin's granuloma, listed as being in the ninth decade of life. This rarity of Hodgkin's disease in the very old depends on the fewer survivors in the higher age brackets, as shown by Minot and Isaacs' [7] finding that the decreasing incidence of lymphoblastoma with advancing age roughly parallels the age distribution of the living population. The present case, however, appears to illustrate well that Hodgkin's disease may not even spare women at an age of more than 80 years.

SUMMARY

A case has been described in which Hodgkin's sarcoma involved the uterus of a senile psychotic person 83 years of age.

3. Lewinski, H.: Zentralbl. f. Gynäk. **54**:2824, 1930.
4. Uddströmer, M.: Virchows Arch. f. path. Anat. **289**:486, 1933.
5. Heller, E. L., and Palin, W.: Arch. Path. **41**:282, 1946.
6. Krueger, F. J., and Meyer, O. O.: J. Lab. & Clin. Med. **21**:682, 1936.
7. Minot, G. R., and Isaacs, R.: J. A. M. A. **86**:1185, 1926.

Laboratory Methods and Technical Notes

A PEROXIDASE REACTION IN PARAFFIN SECTIONS

H. B. RITTER, B.A., and J. J. OLESON, Ph.D., PEARL RIVER, N. Y.

FOLLOWING the introduction of the Romanowsky stains, Maximow showed that the delicate neutrophilic granules, which are so distinct in smears and imprint preparations, are indistinct and uncertain in tissue sections.[1] In the best of paraffin sections most neutrophils show merely a "granular appearance" rather than separate individual granules.

Up to the present there is only one fairly reliable way of demonstrating individual neutrophil granules in sections, namely, the using of one of the peroxidase reactions in frozen sections. Sato and Shoji,[2] Graham,[3] Jacoby[4] and others have applied these reactions with and without gelatin embedding.

In the course of studies made on sections of bone marrow, a need arose to show the neutrophilic granules in paraffin-embedded hemopoietic tissues. Since it is known that the peroxidase reaction is completely lost after paraffin infiltration, and since, on the other hand, it is known that the stained granules are stable once the peroxidase reaction has been applied, it occurred to us that the reaction might be used before the start of the embedding process. In this way, it was thought, one might imitate the "en bloc" staining used in the carmine staining of whole pig embryoes or the Golgi methods employed for the central nervous system.

Pencils of the femoral marrow of rats and guinea pigs were obtained by the method of Mayer and Ruzicka.[5] With this technic the medullary tissue is made accessible to the fixative within five minutes after the thigh is opened, and decalcification is avoided throughout.

We used a procedure in which Graham's peroxidase reaction is modified by the neutralization of the formaldehyde fixative and followed this with a routine sequence of embedding and sectioning.

PROCEDURE

Part 1: Peroxidase reaction.

1. Fixation—Twenty-four hours.

95 per cent ethyl alcohol	90 cc.	
37 per cent solution of formaldehyde U. S. P.	10 cc.	p_H approx. 7.0
0.1 normal sodium hydroxide	1 cc.	

2. Wash in running water ten minutes or allow to stand in water one hour.

From the Lederle Laboratories Division, American Cyanamid Company.

1. Maximow, A.: Arch. f. mikr. Anat. **76**:47 and 55, 1910.
2. Sato, A., and Shoji, K. S.: J. Lab. & Clin. Med. **13**:1058, 1929.
3. Graham, G. S.: J. M. Research **35**:231, 1916.
4. Jacoby, F.: J. Physiol. **103**:22P, 1944.
5. Mayer, E., and Ruzicka, A. Q.: Anat. Rec. **93**:213, 1945.

3. Peroxidase reaction—Twenty-four hours.

Alpha naphthol	1 Gm.
40 per cent ethyl alcohol	100 cc. prepared fresh
Superoxol	0.2 cc.

4. Rinse in running water ten minutes or after several rinses, allow to stand in water one hour.

5. Pyronine stain—Three to twenty-four hours.

Pyronine	0.1 Gm.
40 per cent ethyl alcohol	96 cc.
Aniline oil	4 cc.

Part 2: Paraffin embedding process.

6. 80 per cent alcohol	1 hr.
95 per cent alcohol	2 hr.
Absolute alcohol 1	1 hr.
Absolute alcohol 2	1 hr.
Cedarwood oil	Overnight [6]
Xylene 1	20 min.
Xylene 2	20 min.
Paraffin 1	1½ hr.
Paraffin 2	1½ hr.

7. Embed, block and cut at 4 microns.

Part 3: Counterstains (optional).

In addition to the peroxidase reaction, counterstains may be used. The eosinophilic and basophilic granules assume their usual distinctive staining, while the neutrophilic granules remain as individual red dots.

Nuclear stains, e. g., hematoxylin, do not interfere with the visibility of the granules.

Cytoplasmic stains, e. g., eosin, may be used but should be applied as a light background for the red granules.

Romanowsky stains, e. g., Kingsley's,[7] may be used satisfactorily, provided the red component is kept relatively light. In our experience the neutralized formaldehyde fixation permits the use of this type of stain, with good differentiation of all elements. Hemoglobin is well preserved.

SPECIAL POINTS OF EMPHASIS

It is important that the fixation (part 1, step 1) is not extended beyond twenty-four hours. We have not been able to obtain satisfactory results after a longer period. This may be due to the possible production of formic acid. Although this factor does not appear to be disturbing in the frozen section–peroxidase method, a longer washing time is required in that technic and might well be applied here (part 1, step 2).

In our experience the alcohol-formaldehyde fixative (part 1, step 1) gives results superior to those obtained with an aqueous formaldehyde fixative, whether neutralized or not.

We attempted to find out at which step of the paraffin embedding process (part 2, step 6) the peroxidase reaction becomes negative. When

6. This is our routine procedure. Cedarwood oil might well be omitted if a gradual transfer from alcohol to xylene is carried out in some other way.

7. Kingsley, D. M.: Stain Technol. **10**:127, 1935.

the reaction was applied after the tissue had been treated with absolute alcohol, the granules were less distinct; after treatment with xylene, the reaction was negative.[8]

RESULTS

In the thinner and less cellular areas of the paraffin sections the delicate neutrophilic granules appear as individual red dots in a large number of those cells, a result which cannot be obtained with the Romanowsky stains in paraffin or celloidin[9] sections. The presence of one or more granules in any immature cell identifies it with the granulocytic series. Thus this peroxidase procedure distinguishes the granulocytic series from the lymphocytic series at the premyelocyte stage.

In sections which are treated with this peroxidase procedure the eosinophilic and basophilic cells can be distinguished from the neutrophilic by the conspicuous and characteristic size and shape of their granules. However, these coarse granules are equally well demonstrated by any of the Romanowsky methods.

When not counterstained, the section may show a slight pink tinge as a background of the red granules, but this does not interfere with the use of a counterstain.

This peroxidase procedure has been applied to sections of the livers and the spleens of rats and guinea pigs, as well as to sections of their bone marrows. The demonstration of the granules of the hemopoietic cells of these sections has been equally satisfactory. The usual morphologic characteristics and staining properties of the tissues are preserved by the fixative used. Thus it has proved possible to combine the peroxidase procedure with the routine stains of such organs without interfering with other studies of these tissues. Leukemic or suppurative infiltrations of any organ can obviously be treated in the same manner. There is no doubt that the combining of a peroxidase reaction with the paraffin technic will facilitate the differentiating of the myeloid from the lymphoid series of cells in tissues.

SUMMARY

A successful presentation of the neutrophilic granules occurring in paraffin sections of the rat's and the guinea pig's marrow, liver and spleen is accomplished by applying a modification of Graham's peroxidase reaction to freshly fixed tissue prior to the dehydrating and embedding processes.

The preservation of structures and the staining properties of the tissues are not affected by the procedure.

The new procedure is considered as a tool for classifying the hemopoietic cells of the tissues.

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8. It is self evident that the tissue must be carried back to 40 per cent alcohol before the peroxidase procedure is applied.

9. Celloidin is a concentrated preparation of pyroxylin.